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## CANADIAN SUNFLOWER SEED

### I. BUSHEL WEIGHT AS A FACTOR IN GRADING[1]


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## Abstract

To determine the importance of bushel weight as a factor in grading, 51 samples of Sunrise and 34 samples of Mennonite seed were examined for associations between bushel weight and other properties of commercial sunflower seed.

Highly significant correlations of bushel weight with total oil content of the seed, $r = .867^{**}$; percentage kernel, $r = .740^{**}$; percentage oil in the kernel, $r = .795^{**}$; and percentage nitrogen in the kernels, $r = -.467^{**}$ were obtained. Oil yield, estimated on the basis of a cake containing 5% oil and 10% moisture, was also associated with bushel weight, $r = .871^{**}$. The correlation coefficients of bushel weight with iodine value, acid value, and refractive index were not significant.

The present grade specifications fail to take full advantage of bushel weight as a factor in grading sunflower seed since the levels were set too low to be effective. It is suggested that the levels might well be 30 lb. for No. 1, 26 lb. for No. 2, and 22 lb. for No. 3 instead of 24 lb. for No. 1 and 21 lb. for No. 2 and No. 3. The effects of this change are discussed in relation to grading of the two varieties and the establishment of appropriate price spreads between grades.

** *Indicates that the 1% level of significance was attained.*


The commercial production of sunflower seed was undertaken in Canada in the season of 1943 as a wartime measure to alleviate a serious shortage of vegetable oils. Since no extensive body of data on commercial samples was available, it was necessary to set arbitrary grade specifications and price differentials between grades. The situation was further complicated since two varieties, Mennonite and Sunrise, which differ quite markedly in seed characteristics, were grown. To meet this situation four separate classes of seed were designated in the grade specifications, namely, Canada Western Sunrise, Canada Western Mennonite, Mixed Canada Western, and Sample Canada Western. For the first three classes the minimum bushel weight was set at 24 lb. for No. 1 seed and at 21 lb. for No. 2 and No. 3 seed. On this basis the prices of No. 1 Mennonite and No. 1 Sunrise were set at the same level with a graduated scale of discounts for the lower grades and mixtures and for samples grading tough, damp, or wet.


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If sunflower seed is to find a permanent place in Canadian agriculture it is imperative that an adequate and efficient grading system be devised, which will permit an equitable adjustment between grades and market values. Since bushel weight can be easily determined and is at present widely used in grade specifications for other grains, it appeared that an investigation of the relations between bushel weight and the properties of sunflower seed would be of value in this connection.

The present paper contains data showing the interrelation of sunflower properties with special reference to the associations involving bushel weight. This investigation may not provide a final solution to the problem of the place of bushel weight in grading this crop, but it at least indicates that certain improvements in the present specifications can be made.

## Materials

Samples for this investigation were taken for grading purposes from each carlot of sunflower seed inspected at Winnipeg, Man., during the period October, 1943, to March, 1944. After an official grade has been assigned, the remainder of the sample, approximately 1 lb., was forwarded to the Oil Seeds Laboratory where the additional data reported in this paper were obtained. Distribution of the number of samples according to variety and grade is shown in Table I.

TABLE I

NUMBERS OF SAMPLES ANALYSED ACCORDING TO VARIETIES AND OFFICIAL GRADES

| Grade | Sunrise | | | Mennonite | | |
|---|---|---|---|---|---|---|
| | 1 C.W. | 2 C.W. | Total | 1 C.W. | 2 C.W. | Total |
| Straight grade | 22 | 9 | 31 | 4 | 2 | 6 |
| Tough | 16 | 3 | 19 | 8 | 6 | 14 |
| Damp | 1 | 0 | 1 | 3 | 8 | 11 |
| Moist | 0 | 0 | 0 | 0 | 3 | 3 |
| Total | 39 | 12 | 51 | 15 | 19 | 34 |

Since the Sunrise variety is a week to 10 days later in maturing than Mennonite it was grown almost exclusively in southern Manitoba and all samples of this variety received were from that area. The Mennonite samples were produced in western Manitoba and eastern Saskatchewan. It will be noted from Table I that Sunrise shows a much higher proportion of 1 C.W. samples than Mennonite. This is due in part to a lower bushel weight for Mennonite but is also due to irregularities in seed size. It has been noted (3) that the interaction between seed size and plant spacing is much greater for Mennonite than it is for Sunrise and hence any unevenness in stand would result in a more ragged sample from the Mennonite variety. Furthermore, this variety has a larger seed, which tends to give more cracked and dehulled

seeds in harvesting. The much greater proportion of tough, damp, and moist grades found in Mennonite seed was due mainly to unfavourable harvesting weather prevailing in the areas where this variety was grown.

To prevent the samples from spoiling due to mould growth, they were air-dried after grading and all subsequent work in this laboratory was done with seed at a moisture content in equilibrium with the laboratory atmosphere.

## Methods

*Cleaning*

The samples were received as taken from the car and contained a quantity of stems, leaves, flower parts, dehulled seed, and other grains. To remove this material the seed was first put through a small fanning mill using No. 24 and No. 12 screens for Mennonite and No. 18 and No. 10 screens for Sunrise. This removed most of the trash, loose hulls, and small meats. However, considerable quantities of dehulled seed, barley, oats, and large wheat remained in the samples. These were removed by passing the samples over a small specific gravity table constructed in the laboratory. After these operations the seed was free of all materials classed as dockage and in addition contained none of the larger hulled meats that remained on the smaller screens and under grading specifications are permitted in the sample. By this procedure all samples of Sunrise degraded on account of cracked and hulled seed were raised to 1 C.W. standards. Similar considerations apply to the Mennonite samples with the exception that a number of these would fail to attain 1 C.W. standard owing to lack of uniformity in seed size and a few would fail to attain the required bushel weight of 24 lb.

*Weight per Bushel*

This value was determined using a standard 1 pint measure and a Cox funnel. Measurements were made in duplicate on the thoroughly air-dried samples. It is well known that for most grains the moisture content of the sample influences the bushel weight. Data showing this effect for sunflower seed are presented in a later section.

*Percentage Kernel*

Dehulling seed by hand is slow and tedious and the size of samples that can be used is so small that results are not reliable. To overcome this difficulty a mechanical huller was used to handle larger samples and obtain sufficient meats and hulls for other determinations. The huller was a small model of the rotary impact type that was designed and constructed in the laboratory. Separation of meats and hulls was secured by passing the material over a fine screen and into an air blast. Any seed not hulled in the first pass through the huller was separated by hand and returned to the huller and subsequently fanned. The fine material that passed through the screen consisted of small particles of meats and hull and was separated by reducing the air flow in the fanning mill to a minimum and fanning the material.

Using this equipment and procedure 100 gm. samples of seed were dehulled. The average total weight of hulls and meats recovered amounted to 99 gm. and with a slight amount of hand-picking the meats were recovered in a hull-free condition.

It is apparent that a determination of this type is not strictly quantitative since small particles of meats are removed with the hulls in the fanning operation. However, these losses were small and it is evident that similar losses cannot be avoided in commercial handling. As a check on the efficiency of the operation, recovery of the oil from the seed was checked from analytical data. For Sunrise, with an average total oil content of 30.1 lb. per 100 lb. of seed, dry basis, the oil recovery estimated from the average kernel percentage and oil content of the kernels was 29.9 lb. This corresponds to a loss of 0.20 lb. of oil or 0.38 lb. of meats per 100 lb. of seed on a dry basis. In Mennonite seed this loss was 1.1 lb. of oil or 2.15 lb. of meats per 100 lb. of seed.

This difference between varieties is accounted for by the difference in kernel characteristics. In Sunrise the kernels are round and, in hulling, do not break to the same extent as the kernels of Mennonite, which are flat and thin. Furthermore, in Sunrise a poorly filled seed still retains its characteristic kernel shape while in Mennonite the kernel tends to be of normal area but of paper thinness. These thin kernels break very readily and are extremely difficult to separate from the hulls.

*Analytical Methods*

The methods for oil content, moisture, nitrogen, refractive index, acid value, and iodine value were essentially those previously reported in connection with studies on flax (4).

## Results

Analytical data on the whole seed, the kernel, and the oil are summarized in Table II as means over all samples of each variety. In addition, the range of values obtained and the standard deviation from the means are given to show the variations within each variety for the properties listed.

It is evident from these data that Sunrise seed is definitely higher in total oil content, percentage kernel, bushel weight, and percentage oil in the kernels than Mennonite seed. The slight difference in total nitrogen in the kernels, 0.11%, is of doubtful significance since the samples were not from strictly comparable environments. The oil properties, iodine value, acid value, and refractive index, are virtually the same for both varieties. It would appear that the variety Sunrise is definitely superior to Mennonite for oil production and that the quality of the oil from the two varieties is similar.

If a clean separation of meats and hulls is obtained and it is assumed that a cake having an oil content of 5% and a moisture content of 10% is produced, the relative oil and meal yields can be computed. This was done and the data are recorded in Table III as pounds of oil and meal per 100 lb. of seed on a dry basis. The estimated protein content of the cake is also listed in

TABLE II

MEAN VALUES FOR SEED, KERNEL, AND OIL PROPERTIES OF 51 SAMPLES OF SUNRISE AND 34 SAMPLES OF MENNONITE SUNFLOWER SEED

| Material | Property | Sunrise | | | Mennonite | | |
|---|---|---|---|---|---|---|---|
| | | Mean | Range | S.d.* | Mean | Range | S.d.* |
| Whole seed | Oil, % dry basis | 30.1 | 7.9 | 1.28 | 27.3 | 8.2 | 2.02 |
| | Kernel, % dry basis | 56.1 | 7.5 | 1.33 | 51.2 | 17.1 | 3.64 |
| | Bushel weight, lb. | 32.4 | 10.4 | 1.83 | 27.7 | 8.9 | 2.65 |
| Kernel | Oil, % dry basis | 53.3 | 10.5 | 1.84 | 51.2 | 8.8 | 2.37 |
| | Nitrogen, % dry basis | 4.52 | 1.67 | 0.28 | 4.63 | 1.39 | 0.34 |
| Oil | Iodine value, Hanus | 137.1 | 6.6 | 1.03 | 137.3 | 4.3 | 1.19 |
| | Acid value, mgm. KOH | 0.28 | 0.55 | 0.09 | 0.24 | 0.31 | 0.08 |
| | Refractive index, $n_D^{25}$ | 1.47375 | 0.00079 | 0.00011 | 1.47381 | 0.00064 | 0.00013 |

* *S.d. = Standard deviation, i.e., 68% of the samples differ by less than this amount from the mean value.*

TABLE III

DATA, SHOWING ESTIMATED YIELDS OF OIL AND CAKE FROM 100 LB. OF DRY SUNFLOWER SEED, COMPUTED ON THE BASIS OF A HULL-FREE CAKE CONTAINING 5% OIL AND 10% MOISTURE

| Property | Sunrise | | | Mennonite | | |
|---|---|---|---|---|---|---|
| | Mean | Range | S.d. | Mean | Range | S.d. |
| Oil, lb. per 100 lb. dry seed | 28.3 | 6.6 | 1.41 | 24.8 | 12.0 | 2.49 |
| Cake, lb. at 5% oil and 10% moisture | 30.9 | 6.5 | 1.21 | 29.3 | 7.7 | 2.04 |
| Protein, % in cake at 5% oil and 10% moisture | 51.3 | 10.1 | 2.24 | 50.4 | 10.5 | 2.29 |

the last line of the table. It is again evident that Sunrise is superior to Mennonite in oil and meal yields and tends to yield meal of slightly higher protein content.

It should be noted that the values given in this table are in pounds of oil and meal per 100 lb. of seed on an oven-dry basis. These data may be readily converted to any desired original moisture content. For example, at 9.5% moisture the average yields of oil and meal for Sunrise would be 25.6 and 28.0 lb., respectively, while for Mennonite the values would be 22.4 and 26.5 lb. In a similar manner yields may be estimated at higher moisture levels for the computation of appropriate price spreads between tough, damp, moist, and wet seed.

As indicated in a previous section all determinations were made on seed air-dried in the laboratory. The quantitative data were calculated on a moisture free basis for comparative purposes, and values at any given moisture level

can be readily estimated. The data in Table IV show the moisture level prevailing in the whole seed, the kernels, and the hulls at the time of analysis and are reproduced here to illustrate differences in the moisture distribution within the seed. Differences between varieties in the moisture level of the

TABLE IV

MEAN AIR-DRY MOISTURE LEVELS OF WHOLE SEED, KERNELS, AND HULLS OF SUNRISE AND MENNONITE SUNFLOWER SEED

| Variety | Moisture, % | | | |
|---|---|---|---|---|
| | Whole seed | Kernels | Hulls | Difference, hulls−kernels |
| Sunrise | 4.54 | 4.13 | 5.28 | 1.15 |
| Mennonite | 4.83 | 4.22 | 5.87 | 1.65 |
| Difference between varieties | 0.29 | 0.09 | 0.59 | 0.50 |

whole seed and the kernels would be expected because of the higher oil content of Sunrise seed. The differences between hulls and kernels would also be expected for similar reasons. The hulls from Mennonite show evidence of a higher moisture holding capacity than those from Sunrise. The reason for this is not immediately apparent, but it may be due to the greater density of the Sunrise hulls.

Bushel weights were determined at the moisture levels shown in Table IV but it is impossible to correct these values to dry basis by the usual methods of computation. To secure some idea of variations in bushel weight with changes in moisture level, eight samples of each variety were selected and the bushel weights were determined at different moisture levels. These moisture levels were obtained by storing the samples in wire mesh cages in a cabinet equipped with temperature and humidity controls, till equilibrium moisture was established. Required relative humidities were estimated from the data of Larmour, Sallans, and Craig (1). Data for the means of eight samples of each variety are presented graphically in Fig. 1. For Sunrise, bushel weight decreased by 0.13 lb., and for Mennonite by 0.20 lb., for each increment of 1% in moisture level. It will also be noted from Fig. 1 that as the humidity was increased to obtain higher moisture levels the differences in moisture level between the two varieties noted in Table IV also increased.

This suggests a difference in the hygroscopic equilibrium curves for the two varieties and may indicate that the safe moisture limit for storing Sunrise seed should be slightly lower than the corresponding value for Mennonite seed (1, 2, and 6).

While it appears that grain that has been wetted and dried does not return to exactly the same bushel weight (7), the data illustrated in Fig. 1 provide an estimate of the effects of changes in moisture content. If these regressions are applied to the data in Table II, the average bushel weights of Sunrise and Mennonite at 9.5% moisture would be 31.7 lb. and 26.9 lb., respectively.

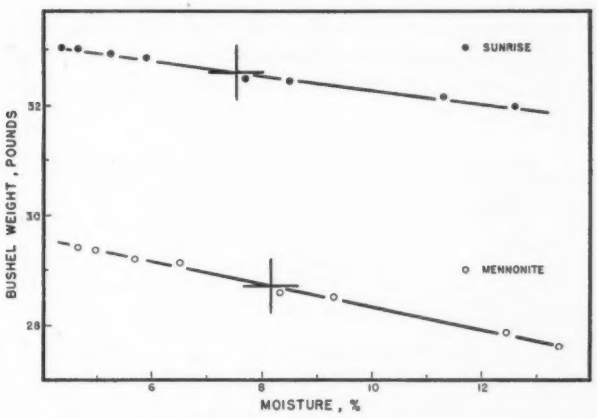


FIG. 1. *Regression of bushel weight on the moisture level of sunflower seed.*

## Correlation Studies

Of the properties listed in Tables II and III only bushel weight was used in establishing grades for sunflower seed. To determine to what extent the other properties of primary interest to crushers are related to bushel weight, a number of correlation coefficients were determined. These coefficients were computed for the varieties separately but, since the varietal coefficients were similar in sign and magnitude, total coefficients over both varieties are reported in this paper. The total simple correlation coefficients among whole seed and kernel properties are shown in Table V. It is evident that highly

TABLE V

SIMPLE CORRELATION COEFFICIENTS COMPUTED OVER BOTH VARIETIES AMONG WHOLE SEED AND KERNEL PROPERTIES OF SUNFLOWER SEED

| Property | Oil, % in whole seed | Kernel, % of whole seed | Oil, % in kernel | Nitrogen, % in kernel |
|---|---|---|---|---|
| Bushel wt., lb. | .867** | .740** | .795** | −.467** |
| Oil, % in whole seed | | .761** | .815** | −.530** |
| Kernel, % of whole seed | | | .525** | .423** |
| Oil, % in kernel | | | | −.735** |

NOTE:—*Throughout the tables, ** indicates that the 1% level and * that the 5% level of significance has been attained.*

significant associations exist among bushel weight, oil content of the whole seed, percentage kernel in the whole seed, oil content of the kernels, and nitrogen content of the kernels. As bushel weight increases the percentage kernel increases and along with this effect the oil contents of both the whole seed and the kernels also increase. This is accompanied by a significant decrease in the nitrogen content of the kernels.

To estimate the significance of bushel weight in controlling relations among the other properties, the partial coefficients, independent of bushel weight, shown in Table VI were computed. From these data it is apparent that

TABLE VI

PARTIAL CORRELATION COEFFICIENTS, INDEPENDENT OF BUSHEL WEIGHT, AMONG WHOLE SEED AND KERNEL PROPERTIES OF SUNFLOWER SEED

| Property | Kernel, % of whole seed | Oil, % in kernel | Nitrogen, % in kernel |
|---|---|---|---|
| Oil, % in whole seed | .353** | .417** | −.283* |
| Kernel, % of whole seed | | −.155 | .130 |
| Oil, % in kernel | | | −.677** |

common associations with bushel weight make up the greater part of the association of total oil content of the seed with percentage kernel, and oil and nitrogen contents of the kernels. Associations of kernel percentage with oil and nitrogen contents of the kernels are apparently due solely to common relations of these properties with bushel weight. From the nature of the negative association between oil and nitrogen contents of the meats it is not surprising that this relation persists even when common associations with bushel weight are eliminated by computing the partial coefficient. It thus appears that bushel weight is of fundamental importance in determining the composition of sunflower seed and hence may be of great value in setting up a satisfactory grading system.

The relations between bushel weight and the properties related to oil quality were examined and the simple correlation coefficients listed in Table VII show that bushel weight gives no indication of oil quality. Quality

TABLE VII

SIMPLE CORRELATION COEFFICIENTS AMONG THE PROPERTIES, BUSHEL WEIGHT AND IODINE VALUE, ACID VALUE, AND REFRACTIVE INDEX OF SUNFLOWER OIL

| Property | Iodine value | Acid value | Refractive index |
|---|---|---|---|
| Bushel weight | −.052 | .041 | −.264* |
| Iodine value | | −.473** | .779** |
| Acid value | | | −.376** |

appears to depend on factors such as maturity of the seed and conditions of storage. Associations among iodine value, acid value, and refractive index of the oils are to be expected in view of previous reports on linseed oil (4 and 5) and will not be discussed in this paper.

From the crushers' viewpoint the association of bushel weight with yields of oil and meal and protein content of the meal are of primary interest. The

correlation coefficients for these properties are shown in Table VIII. The most striking feature of these data is the close positive association between bushel weight and the estimated oil yield. The coefficient, $r = .871^{**}$,

TABLE VIII

SIMPLE CORRELATION COEFFICIENTS AMONG BUSHEL WEIGHT, OIL YIELD, CAKE YIELD, AND PROTEIN CONTENT OF THE MEAL AT 5% OIL AND 10% MOISTURE

| Property | Oil yield | Cake yield | Protein, % in cake |
|---|---|---|---|
| Bushel weight | .871** | .287** | .132 |
| Oil yield | | .367** | .206 |
| Cake yield | | | .411** |

indicates that over the 85 samples tested, irrespective of variety, 75% of the variance in oil yield can be accounted for by differences in the bushel weights of the samples. There is also a slight tendency for samples of high bushel weight to give high yields of cake. High bushel weight, therefore, provides an indication of high oil and cake yields in processing. However, bushel weight gives no significant indication of the protein content of the cake.

Of the associations discussed in the preceding paragraphs those of total oil content and oil yield with bushel weight are of the greatest interest in considering grading problems and for this reason they were studied in greater detail. The data were subjected to analyses of residual variance for varietal regressions of oil content and oil yield on bushel weight. The results of these analyses are shown in Table IX. It is evident from these data that varietal

TABLE IX

ANALYSES OF RESIDUAL VARIANCE FOR VARIETAL REGRESSIONS OF TOTAL OIL CONTENT AND ESTIMATED OIL YIELD ON BUSHEL WEIGHT OF SUNFLOWER SEED

| Variance due to: | Degrees of freedom | Mean squares | |
|---|---|---|---|
| | | Oil content | Oil yield |
| Differences among varietal regression coefficients | 1 | 0.4854 | 0.0698 |
| Deviations of varietal means from average regression | 1 | 0.4862 | 1.3037 |
| Residual deviations from individual varietal regressions | 81 | 1.1616 | 1.6275 |
| Total regression coefficient | | 0.578 | 0.700 |

regressions do not differ significantly and that varietal means do not deviate significantly from the average varietal regressions for either of these associations. Hence, the relations can be adequately represented by the total

** *Indicates that the 1% level of significance was attained.*

regressions. To present actual data and to clarify these relations, scatter diagrams are presented in Figs. 2 and 3. In these figures values for the two varieties are differentiated, the means for each variety and the total regressions are shown. The individual varietal regressions have been omitted since they are practically coincidental with the total regressions.

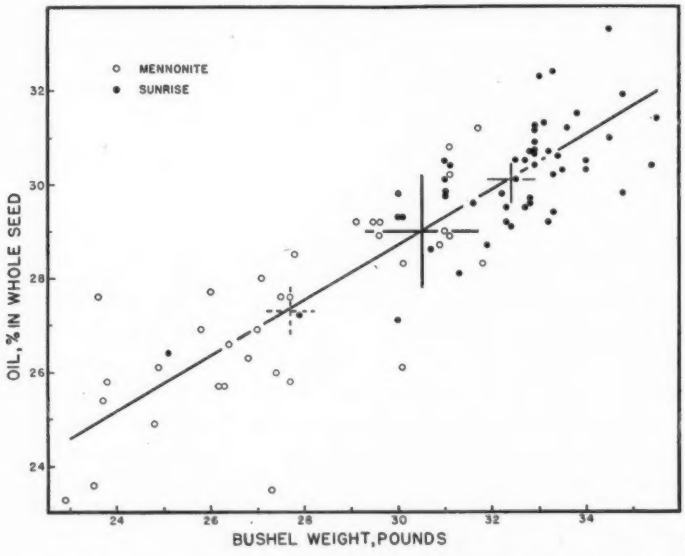


FIG. 2. *Scatter diagram showing the relation between oil content of sunflower seed and bushel weight.*

It should be noted that the total oil content of the seed shows an average increase of 0.578 units for each unit increase in bushel weight, while for oil yield this increase is 0.700 units. This reflects the greater oil loss from poorly filled kernels due to the increased breakage of meats and losses of light seed during hulling and fanning operations on seed of low bushel weight.

## Discussion

The data presented in the preceding sections show that bushel weight is a good indication of the oil content of sunflower seed and of the returns in oil and meal that may be expected. Furthermore, this relation is the same for both Mennonite and Sunrise seed. If individual samples are considered, exceptions will be found as indicated in the scatter of the points in Figs. 2 and 3, but if a large bulk of seed made up of a number of individual parcels is considered it would appear that bushel weight should furnish a reasonably accurate index of the value of the seed for crushing purposes.

An examination of the scatter diagrams, Figs. 2 and 3, indicates that only five out of 85 samples fell below 24 lb., the minimum bushel weight for No. 1

seed, and not a single sample fell below the minimum level, 21 lb., for No. 2 seed. It appears, therefore, that while bushel weight is a fairly good indication of the value of sunflower seed, the levels for bushel weight were set too low for the present grade specifications to reflect this value. This is particularly true of the Sunrise variety, which showed an average bushel weight

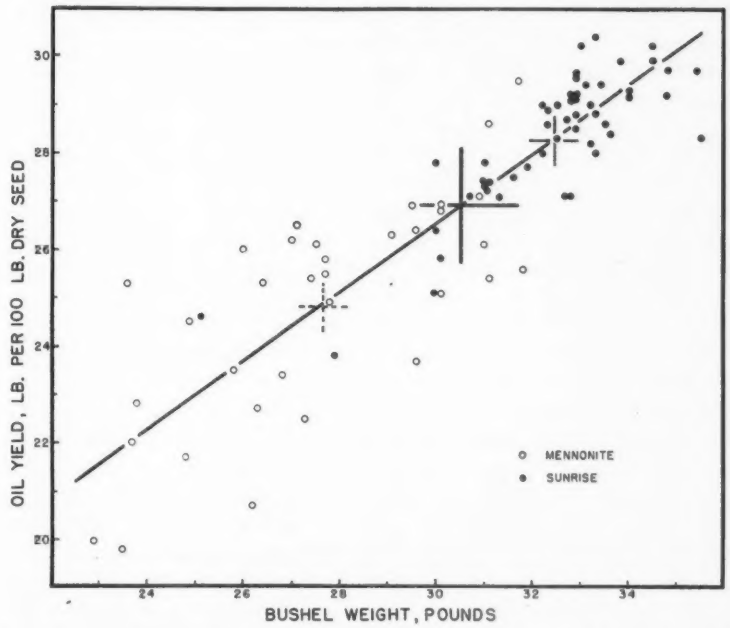


FIG. 3. *Scatter diagram showing the relation between oil yield, estimated for a cake containing 5% oil and 10% moisture, and bushel weight of sunflower seed.*

of 32.4 lb. at a moisture level of 4.5% (estimated as 31.7 lb. at 9.5% moisture). Even Mennonite seed had an average bushel weight of 27.7 lb. (estimated as 26.9 lb. at 9.5% moisture) which is considerably in excess of the minimum bushel weight for No. 1 seed.

There will undoubtedly be variations in the general level of bushel weight with different seasons and it is, therefore, impossible to recommend a definite and fixed gradation on the basis of a single crop. However, it should be possible to set tentative values that can be revised as greater experience is gained in handling sunflower seed. If it is assumed that the minimum bushel weights should be 30 lb. for No. 1, 26 lb. for No. 2, and 22 lb. for No. 3, the distribution of the samples on this basis can be estimated. This was done with the following result:—Sunrise: No. 1, 45; No. 2, 5; No. 3, 1; Mennonite: No. 1, 7; No. 2, 15; No. 3, 12.

It is evident that a division such as this would tend to place the Sunrise variety in the top grades and to degrade Mennonite on the basis of bushel weight. If grades are to give any indication of the value of sunflower seed, this would be desirable since the data indicate that the average oil yield for Sunrise was 28.3 lb. and for Mennonite 24.8 lb. per 100 lb. of dry seed. Taking oil yield as the sole criterion of value this would indicate that the prices of Sunrise and Mennonite seed should stand in the ratio of 1.0 to 0.88 on the basis of variety. It is thus obvious that if the same prices are paid for Sunrise and Mennonite a processor handling Sunrise seed can produce oil at considerably lower cost than one who handles Mennonite seed. If, however, bushel weights were specified as suggested in the preceding paragraph the prices of grades Nos. 1, 2, and 3 should stand in the ratio of 1.00, 0.88, and 0.78 on the simple criteria of bushel weight and oil yield irrespective of variety. Obviously, a single property such as bushel weight cannot be used as the only standard in defining grades; such factors as 'soundness', damaged seed, and uniformity of seed size, which influence oil quality and cleaning and handling costs cannot be neglected. However, it is virtually impossible to assess the importance of these factors from laboratory tests on small samples and it appears that they must be worked out under commercial conditions.

While this investigation indicates that the association of oil yield with bushel weight is the same for both Sunrise and Mennonite seed, there still appears ample justification for keeping these varieties separate and for degrading mixtures. The Mennonite seed is considerably larger and of lower density than Sunrise; hence, if the varieties were mixed, difficulties that would raise handling costs in cleaning the seed would be encountered. Furthermore, in dehulling the seed it is advantageous to have a uniform seed size as this tends to give less breakage of kernels and cleaner separations of kernels and hulls. If the two varieties were mixed, greater oil losses in hulling would occur since Mennonite seed has a greater tendency to break in hulling, while Sunrise seed has a denser hull, which requires higher air velocities for separation of kernels and hulls. With mixtures these tendencies would result in greater losses of meats than if the varieties were handled separately.

It is obvious from the preceding discussion that the establishment of grade specifications and price relations is a complex problem and many factors can only be resolved on the basis of commercial experience. However, the data presented in this paper indicate that the single property, bushel weight, provides a fair index of the actual oil yield that can be obtained in crushing. Therefore, a better application of bushel weight specification for sunflower grades would result in a grading system more indicative of the relative commercial value of different parcels of grain.

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and the members of his staff who collected the samples for this investigation. They also appreciate the careful analytical work of Mr. G. D. Sinclair, Laboratory Assistant, in connection with this study.

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# DRIED WHOLE EGG POWDER

## XVI. RELATIVE STABILITY OF POWDERS OF DIFFERENT QUALITY[1]

By M. W. Thistle[2], W. Harold White[3], D. A. Fletcher[4], and Jesse A. Pearce[2]


### Abstract

When stored at 24° C. (75° F.) for one month, samples of dried whole egg powder of varying initial qualities, collected from 10 Canadian plants over a period of six months, developed fluorescent materials at much the same rate. Powders from two of the plants, heat-treated to provide good, questionable, and definitely poor samples, were stored at 24° C. for periods up to four months. Some of the observed differences in stability were attributable to different moisture contents; but within plants, good and poor quality powders deteriorated at the same rate. In a third experiment, mixtures of good and poor quality powders having a similar moisture content also deteriorated at the same rate. It is concluded that the rate of quality deterioration in egg powders, as determined by fluorescence changes, is independent of the initial quality.


### Introduction

The question has sometimes been asked: do egg powders of varying initial quality deteriorate differentially? This has considerable practical significance in that if good quality powders deteriorate at a relatively rapid rate, some of the advantage of excellent initial quality might be lost during shipment or storage. Although information is available on the stability of egg powder (2, 3, 7, 8, 9), this particular point does not appear to have received detailed attention. The present study was designed to answer the question.

### Materials and Methods

In these laboratories, during 1942, a survey was made of the quality of all the dried egg powder produced in Canada. At each egg-drying plant samples were taken from each carlot of powder intended for export to Britain, and their initial quality determined. Duplicates of certain samples were stored for a month at 24° C. (75° F.) in order to assess the relative stability of powder produced at different seasons and in different plants.

In studies on the effects of heat treatment it was found that dried egg was susceptible to damage by heat at even slightly elevated temperatures: samples from two Canadian plants were heat-treated for five days at 26.7°, 35.0°, and 43.4° C. (80°, 95°, and 110° F.), as described in a previous communication

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(8). These heat-treated samples also were stored at 24° C. for periods up to four months in order to obtain information on their subsequent stability.

Further information was sought by measuring the relative stability of mixtures of poor and good quality material. Egg powder having a fluorescence value (4) of 60 was thoroughly mixed at 0, 1, 5, 15, and 30% levels with powder having a fluorescence value of 18. Uniform moisture contents (from 3.5 to 3.6%) were obtained in the mixed samples by permitting them to stand in a closed chamber at 4.4° C. (40° F.). Samples of the mixed powders were stored at 26.7° and 37.8° C. (80° and 100° F.) for periods of 3, 10, 17, 24, and 31 days. Initial fluorescence values of the treated powders ranged between 18 and 27, and were approximately proportional to the amount of poor powder added.

Quality was assessed by means of potassium chloride (6) and fluorescence values (4, 5, 6), chosen for present purposes as being most sensitive of the objective tests available. Indeed, the fluorescence test has been shown to be remarkably sensitive to heat damage in dried eggs (8) even at temperatures as low as −40° C. (7). Since moisture content has been shown to have important effects on the stability of dried egg (1, 2, 9) the total volatile content (6) was also noted.

## Results

*Survey Material*

The results are presented in Table I. It is apparent that initial quality (as measured by fluorescence) was lower during July and August, the hottest months: the defect in commercial practice responsible for this lower quality was rectified in 1943 by the installation of devices for cooling the powder as it left the drier. Stability was poor in July (average of only five samples) but otherwise it appears that seasonal samples deteriorated at about the same rate under the storage conditions used. Also samples from different Canadian plants all deteriorated at about the same rate.

The influence of moisture content, under these conditions, was rather small.

The potassium chloride values, while more variable than the fluorescence measurements, present essentially the same information.

*Heat-Treated Samples*

The results are shown in Fig. 1.

The powder from Plant I deteriorated at a slower rate than that from Plant II: this difference is considered to be due primarily to the fact that the powder from Plant I contained 3.5% moisture while that of Plant II contained 5.6%. It has been shown that the stability of egg powder decreases with increase in the moisture content (9).

However, within plants the curves for good, intermediate, and poor powders possess approximately the same slope, indicating that if a uniform moisture content is attained, all powders in the edible range of quality deteriorate at about the same rate.

TABLE I

MEAN VALUES FOR QUALITY MEASUREMENTS OF DRIED EGG SAMPLES FROM ALL CANADIAN PLANTS, COLLECTED FROM JULY TO DECEMBER, 1942, BEFORE AND AFTER STORAGE AT 24° C. FOR ONE MONTH

| —— | No. of samples | Moisture content, % | Potassium chloride value, % | | Fluorescence value, units | |
|---|---|---|---|---|---|---|
| | | | Initial | Final | Initial | Final |
| *Means, averaged over all plants* | | | | | | |
| Month of collection | | | | | | |
| July, 1942 | 5 | 4.6 | 66 | 50 | 30 | 46 |
| August | 29 | 4.3 | 65 | 53 | 29 | 35 |
| September | 30 | 4.6 | 67 | 54 | 23 | 35 |
| October | 30 | 4.0 | 74 | 62 | 22 | 30 |
| November | 26 | 4.0 | 70 | 62 | 22 | 32 |
| December | 30 | 3.8 | 69 | 62 | 23 | 29 |
| *Means, averaged over all sampling times* | | | | | | |
| Plant No. | | | | | | |
| 1 | 25 | 4.5 | 68 | 57 | 25 | 33 |
| 2 | 20 | 3.9 | 74 | 62 | 22 | 30 |
| 3 | 11 | 3.8 | 73 | 66 | 20 | 26 |
| 4 | 2 | 5.0 | 63 | 51 | 26 | 36 |
| 5 | 24 | 4.3 | 68 | 52 | 29 | 40 |
| 6 | 11 | 4.4 | 70 | 65 | 21 | 31 |
| 7 | 40 | 4.2 | 64 | 53 | 24 | 32 |
| 8 | 10 | 3.2 | 74 | 67 | 21 | 30 |
| 9 | 6 | 4.5 | 78 | 67 | 19 | 30 |
| 10 | 2 | 4.9 | 73 | 73 | 22 | 31 |

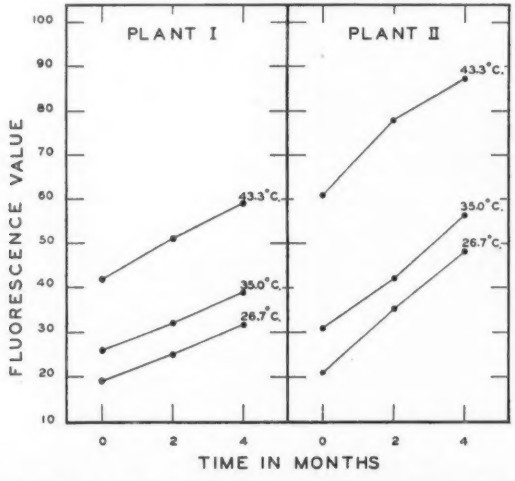


FIG. 1. *Relative stability of heat-treated egg powders stored at 24° C.*

*Mixtures of Poor and Good Powders*

The results are plotted in Fig. 2. The shapes of the curves are similar, indicating that samples with differing initial quality all exhibited similar behaviour under accelerated storage conditions. The small differences in slope were subjected to statistical analysis to ascertain their significance. Table II shows that the interactions between initial quality and storage time, and between initial quality and storage temperature, both failed to attain statistical significance. Hence the addition of low quality powders had no demonstrable effect on the rate of deterioration.

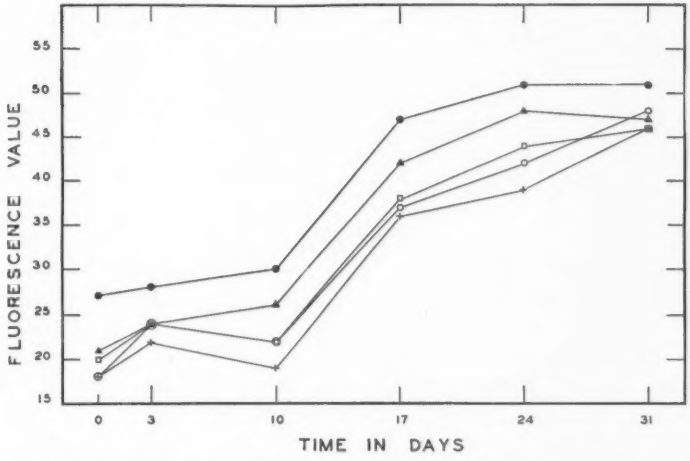


FIG. 2. *Relative stability of mixtures of poor and good quality egg powders, averaged over storage temperatures of 26.7° and 37.8° C. Percentages of poor powder added:* + = 0; ○ = 1; □ = 5; ▲ = 15; ● = 30.

TABLE II

ANALYSIS OF VARIANCE OF THE RELATIVE STABILITY OF MIXTURES OF POOR AND GOOD QUALITY EGG POWDERS

| Source of variance | Degrees of freedom | Mean square |
|---|---|---|
| Initial quality × time | 16 | 11.4 |
| Initial quality × temperature | 4 | 9.4 |
| Initial quality × time × temperature (error) | 16 | 9.8 |

## Conclusion

The evidence presented shows that when the moisture content was similar, good quality egg powders did not deteriorate at a faster rate than powders of lower quality in the edible range.

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# PACKAGING

## I. WATER-VAPOUR RESISTANCE OF CELLULOSE-BASE CONTAINERS[1]

By A. H. Woodcock[2], Marion G. Chapman[3] and Jesse A. Pearce[4]


### Abstract

Lacquers and resins coated on paper stocks were less effective water-vapour barriers than waxes. Wax coatings became more effective as the density of the base stocks increased. A flexible wax compound at 40 lb. per ream of 500 sheets, 24 by 36 in., was found most satisfactory for coating "Cellophane."

Rectangular containers were found more suitable than cylindrical containers. Wax dipping followed by an overwrap produced water-vapour–resistant packages, but the bag-in-box type, using wax-coated Cellophane as liner, was also effective. Pouch-type liners were favoured.

Bag-in-box type packages using wax-coated Cellophane as the liner bag were developed for use on dried egg powder, and a wax-impregnated, wax-coated outer container was devised for Army Mess Tin Ration Kits.

Mean moisture gain plus twice the standard deviation was used as a merit factor in distinguishing between packages.


### Introduction

Shortages of tin plate, rubber, and shipping space have increased the importance of dehydrated foods. Dried foods generally keep better if their moisture content is maintained at or near the original low level: this raised the problem of suitable containers for their protection, and reasonably water-vapour–resistant packages had to be provided from available packaging materials, such as paper products and waxes. The urgency of war demanded an early solution of the problem.

Other factors were considered but the present study deals primarily with water-vapour permeability (W.V.P.). Tests on a wide variety of commercially available materials, as received, after folding, and when made into packages, resulted in observations on materials and package design that are believed to be of some general value and are therefore reported here. Since only comparative values of currently used stocks are of practical concern, no attempt has been made to convert results to unit thicknesses of base stock or coatings.

### Methods

A vapometer method of measuring W.V.P., while none too reliable (1), provided simple and rapid comparative measurements on the various stocks,

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both as received and after folding through 180° over the rounded edge of a metal plate 1 mm. thick. Two folds at right angles were made. Thus the better types of water-vapour barriers could be selected for fabrication into experimental packages.

Cylindrical and rectangular packages, each containing 60 gm. of calcium chloride, were placed in a test chamber operating at 80° F. and 85% relative humidity, resulting in a vapour pressure difference (V.P.D.) of 22.4 mm. of mercury. W.V.P. was measured by following the gain in weight. Gains are recorded as the averages of six packages of each type. Dummy packages were included in order to correct for sorption by the packaging materials.

Since one phase of the investigation was concerned with the development of a package suitable for dried egg, further tests were made on packages filled with dried egg powder and stored in the test chamber (V.P.D. approximately 14 mm. of mercury).

Another phase of the investigation dealt with a container for Army Mess Tin Ration Kits: a suitable moisture barrier had to be devised for the outside of the package, since some of the contents is packed in tin plate that might destroy an inner barrier.

*Merit Factor*

The use of mean moisture gain as a measure of package merit is not altogether satisfactory, since 50% of the packages can be expected to gain more moisture than represented by the mean value. The maximum moisture gain is also a poor criterion of package quality since it is usually dependent on a single package. A value descriptive of the great majority of the packages was felt to be desirable, i.e. the mean plus some measurement of variability. In consequence a numerical value, "merit factor," has been used in these laboratories as a criterion of package quality.

The merit factor is defined as the mean plus twice the standard deviation. Theoretically, for large normally distributed populations, 2.5% of the members should exceed this value. Since the number of packages studied is usually small, the merit factor would probably be less reliable than this. In 281 measurements, 11 packages (4%) exceeded the merit factor; in an additional 100 measurements, 5% exceeded the merit factor. Both curves indicated approximately normal distribution. Hence the merit factor might be used as an indication of the protection afforded by approximately 95% of the packages when small numbers are considered.

The merit factor might be used as a standard of reference for commercially available package types if standardized for temperature, water-vapour pressures, size of package, and quantity of hygroscopic material, e.g., calcium chloride. Certain limitations would have to be considered, such as the possibility of badly skewed curves.

## Results

*W.V.P. of Various Packaging Materials*

The results are given in Table I. In general wax-impregnated krafts were less effective than wax-coated krafts; lacquers and resins provided about equal protection and were less effective than waxes; and wax coatings became more effective as the density of the base stocks increased. In the denser stocks, e.g., "Cellophane", wax coating on one side was approximately as effective as wax coatings on both sides and as effective as laminated Cellophane. A commercial firm offered the flexible wax compound found most satisfactory for coating Cellophane (40 lb. of wax per ream of 500 sheets , 24 by 36 in): the term "wax-coated Cellophane" will indicate use of this flexible preparation. W.V.P. measurements after folding showed increases ranging from 1/5 to 60 times depending on the type of material under study.

*W.V.P. of Fabricated Packages*

The results are shown in Table II, and in general support the conclusions drawn from Table I. However, it was found that even under the best conditions, the W.V.P. of completed packages was often 2 to 10 times as high as that of the same material in sheet form.

Of the wax-coated cylindrical containers, the fibre-bodied type was more effective than the all-fibre type. Fibre-bodied containers suffered from failure at the metal-to-fibre seam, and in the all-fibre type it was difficult to obtain a tight closure when inserting the friction-plug lid. Wax-dipping after fabrication improved the fibre-bodied type.

Although cylindrical containers are stronger than rectangular containers, they occupy about 20% more space; however, the relative weakness of rectangular packages can be compensated for by enclosure in a suitable master carton.

Considering rectangular containers, it is evident that an overwrap further reduced the W.V.P. of wax-dipped packages by approximately 50%. The bag-in-box type also produced effective packages. Of the more effective liners, wax-coated Cellophane was preferred since the cost of a second sheet was avoided.

No great difference in effectiveness was evident between wedge and pouch type liners. However, it is considered that there is a danger of leaks developing at the seal around the gusset in the wedge type. Therefore it was believed desirable to use pouch type liners (3).

*Packages for Dried Whole Egg Powder*

A number of package types were tested, using dried egg powder as hygroscopic material (Table III). In addition, differences between and within firms preparing these packages are considered in Table IV, together with an estimate of resistance to handling, i.e., after dropping the packages six times from a height of 30 in. to a cement floor. From consideration of Tables III and IV, and for reasons described elsewhere (3), choice was made of the

## TABLE I

WATER-VAPOUR PERMEABILITY OF FILMS OF VARIOUS PACKAGING MATERIALS AT ROOM TEMPERATURE AND A VAPOUR PRESSURE DIFFERENCE OF APPROXIMATELY 26.3 MM. MERCURY

*(a) Single Sheets*

| Material | | Treatment: None | Impregnated: Wax | Surface coated: Lacquer | Resin | Asphalt | Wax, one side | Wax, two sides |
|---|---|---|---|---|---|---|---|---|
| | | Water-vapour permeability, gm./sq. m./day | | | | | | |
| Stock | | | | | | | | |
| 60 Lb. kraft | plain | — | 742 to 48* | — | 28–34 | 7.0 | 15– 15–17–19 | 0.52–0.54–2.2 |
| | folded | — | — | — | 54–57 | 11 | 130–200–43– | 26 – 33 – 59 |
| 30 Lb. sulphite | plain | — | — | 25–46–52 | — | — | –26 | — |
| | folded | — | — | — | — | — | 14–57 | — |
| 25 Lb. glassine | plain | — | — | 45–97–140 | 34 | — | 4.0–4.3– 7.6 | 4.0 |
| | folded | — | — | — | — | — | –8.4–16 | — |
| 300 M.S.T. Cellophane | plain | 14 | — | — | — | — | 1.2–2.3–4.2 | 2.9 |
| | folded | — | — | — | — | — | –2.6–8.4 | 3.3 |
| 450 M.S.T. Cellophane | plain | — | — | — | — | — | 0.74 | — |
| Pliofilm | plain | 1.8 | — | — | — | — | — | — |
| | folded | 1.9 | — | — | — | — | — | — |

*(b) Laminations*

| Material | | Combined with: 60 Lb. kraft | 30 Lb. kraft | 20 Lb. sulphite | "Greaseproof" paper | 25 Lb. glassine | 300 M.S.T. Cellophane | "Metal-coated" paper | Metal foil and M.S.T. Cellophane |
|---|---|---|---|---|---|---|---|---|---|
| | | Water-vapour permeability, gm./sq. m./day | | | | | | | |
| Bleached Manila board | plain | 7.4 | — | — | 32 | 8.3–11 | — | — | — |
| | folded | — | — | — | 49 | — | — | — | — |
| 60 Lb. kraft | plain | 28–41–43–50† | — | — | — | 20‡ | — | — | — |
| | folded | 48– | — | — | — | 23 | — | — | — |
| 30 Lb. kraft | plain | — | 3.2 | 1.5 | — | — | — | — | — |
| | folded | — | 11.0 | 4.0 | — | — | — | — | 1.4† |
| 25 Lb. glassine | plain | — | — | — | 13 | 1.6–3.1–3.3 | — | — | — |
| | folded | — | — | — | 18 | 5.5–3.7– | — | — | — |
| 300 M.S.T. Cellophane | plain | — | — | — | — | 14 | 1.9–2.9 | 2.0 | — |
| | folded | — | 3.3–3.3 | 4.3 | — | 23 | — | 6.4 | — |

* *Range found after examination of 19 types.*

† *Asphalt as laminating agent, and* ‡ *dextrin glue as laminating agent; all others, wax-base adhesives.*

TABLE II

WATER-VAPOUR PERMEABILITY OF VARIOUS FINISHED PACKAGES SUBJECTED TO 80° F. AND A VAPOUR PRESSURE DIFFERENCE OF 22.4 MM. OF MERCURY

| Description of package | Mean water-vapour permeability, gm./wk. |
|---|---|
| *Cylindrical* | |
| All-fibre, wax-coated inside and out; mean, two types (area of transmission, 38 sq. in.) | 4.0 |
| Fibre-bodied with metal ends, one end friction-plug type (area of transmission, 31.4 sq. in.) | |
| *A.* Untreated, 60 lb. kraft | 9.5 |
| *B.* 60 Lb. kraft and 25 lb. glassine | 2.9 |
| *C.* Asphalt-coated, 60 lb. kraft | 1.2 |
| *D.* Asphalt-laminated paper; mean, two types | 1.2 |
| *E.* Wax-coated, 60 lb. kraft; mean, three types | 0.60 |
| *F.* 60 Lb. kraft, wax-dipped after fabrication | 0.25 |
| *Rectangular* (area of transmission, 50.7 sq. in.) | |
| Wax-dipped | |
| *A.* Dipped once; mean, two types | 0.21 |
| *B.* Dipped twice; mean, two types | 0.16 |
| *C.* Dipped once, then wrapped in 60 lb. kraft; mean, two types | 0.12 |
| Wrapped | |
| *A.* 300 M.S.T. Cellophane | 2.3 |
| *B.* 300 M.S.T. Cellophane over bag-in-box with 300 M.S.T. Cellophane pouch type liner | 0.90 |
| *C.* 60 Lb. kraft, wax-coated both sides, ends redipped after fabrication | 0.13 |
| Bag-in-box (comparison of pouch and satchel liners)* | |
| *A.* Wedge (side seam, wax to Cellophane) | 0.93 |
| *B.* Wedge (side seam, wax to wax) | 0.78 |
| *C.* Pouch | 1.00 |
| Bag-in-box (liner, pouch-type) | |
| *A.* 30 Lb. vegetable parchment, wax coated | 8.1 |
| *B.* 40 Lb. bloodstock, wax coated | 4.8 |
| *C.* 300 M.S.T. Cellophane | 2.3 |
| *D.* Bleached, laminated, 45 lb. glassine, wax-coated | 1.2 |
| *E.* Laminated 300 M.S.T. Cellophane | 0.41 |
| *F.* 300 M.S.T. Cellophane laminated to 25 lb. glassine | 0.16 |
| *G.* 300 M.S.T. Cellophane wax-coated | 0.091 |
| *H.* 300 M.S.T. Cellophane laminated to "metal-coated" paper | 0.070 |

* *Surface area of package approximately 140 sq. in.*

bag-in-box type utilizing wax-coated Cellophane as the liner bag. Two sizes of package were recommended for dried egg powder; a 14 lb. container for restaurant use and a 5 oz. household package. The W.V.P. of the 14 lb. carton when filled with egg powder has been measured as 1.68 gm. per wk. Performance of the 5 oz. carton can be seen in Tables III and IV.

*Fourteen pound container.* This had a Cellophane liner, pouch type, 23 in. deep by 18 in. wide (inside measurements) with a heat-seal on either side of from $\frac{3}{4}$ to 1 in. The bag was made of 450 M.S.Y.T. Cellophane, with a wax coat on the inside. This Cellophane bag was fitted inside a bag made of

TABLE III

WATER-VAPOUR PERMEABILITY THROUGH FINISHED PACKAGES CONTAINING 142 GM. (5 OZ.) OF DRIED EGG POWDER (MOISTURE CONTENT, 5%). SUBJECTED TO 80° F. AND 85% RELATIVE HUMIDITY (V.P.D., APPROXIMATELY 14 MM. OF MERCURY)

| Description of package | Water-vapour permeability, gm./wk. |
|---|---|
| Single wax-dip | 0.119 |
| Single wax-dip then wrapped in 60 lb. kraft | 0.077 |
| Wrapped in 60 lb. kraft, wax-coated both sides; ends redipped and overwrapped in 60 lb. kraft | 0.077 |
| Single wax-dip then overwrapped with 60 lb. kraft, wax-coated both sides and ends redipped | 0.077 |
| Bag-in-box (pouch type liner) | |
| *A.* 300 M.S.T. Cellophane laminated to 25 lb. glassine | 0.126 |
| *B.* Duplex Pliofilm | 0.119 |
| *C.* Wax-coated M.S.T. Cellophane | 0.080 |
| *D.* 300 M.S.T. Cellophane, laminated to "metal-coated" paper | 0.060 |

60 lb. M.F. kraft, satchel bottom style, to give a made-up bag 9 by 9 in. square and $14\frac{3}{4}$ in. high. These were then enclosed in a corrugated carton with inside dimensions 9 by 9 by 10 in. high made from 100% Fourdinier kraft, B-flute board, full meeting flaps, bursting strength 200 lb. Mullen test. The manufacturer's seal consisted of 2 in. Cambric type, since stitches might damage the liner.

*Five ounce household package.* This package was also of the bag-in-carton type: the liner was made of heat-sealed 300 M.S.Y.T. Cellophane with a wax coat on the inside. Outside dimensions were $5\frac{3}{8}$ in. wide by $6\frac{3}{4}$ in. high; inside dimensions were $4\frac{5}{8}$ in. wide by $6\frac{3}{4}$ in. high. This was enclosed in a carton of outside dimensions of 4 in. high by $2\frac{3}{4}$ in. wide by 1 15/16 in. deep. The carton was of flat folding type with overlapping long flaps, made from 0.020 in. board free from reclaim, good bender.

These two packages enclosed in suitable semi-master and master containers, have to date proved satisfactory for shipments of dried egg powder from Canada to Great Britain.

*A Container for Army Mess Tin Ration Kits*

Examination of the contents of the old-style kits after dropping tests showed that many of the packages contained therein had ruptured. From the data in Table II many of these inner packages were redesigned. Some of these have been described (2).

The ration kit contains tin plate packages, so that wax-coated Cellophane would not be satisfactory as a water-vapour barrier for the outer container.

TABLE IV

WATER-VAPOUR PERMEABILITY OF FINISHED PACKAGES (FROM VARIOUS MANUFACTURERS) CONTAINING 142 GM. (5 OZ.) OF DRIED EGG POWDER (MOISTURE CONTENT, 5%) SUBJECTED TO 80° F. AND 85% RELATIVE HUMIDITY WITH AND WITHOUT ROUGH HANDLING. (V.P.D., APPROXIMATELY 14 MM. OF MERCURY)

| Description of package | Manufacturer | Water-vapour permeability | | | |
|---|---|---|---|---|---|
| | | Without handling | | With handling | |
| | | Mean, gm./wk. | Merit factor | Mean, gm./wk. | Merit factor |
| Carton adhesively sealed, wax paper wrapped, heat-sealed, ends dipped in wax, and whole overwrapped with kraft | *A* | 0.200 | 0.272 | 0.297 | 0.397 |
| | *B* | 0.259 | 0.407 | 0.354 | 0.544 |
| | *C* | 0.561 | 0.751 | | |
| | *D* | 0.234 | 0.370 | | |
| | *D* | 0.170 | 0.234 | | |
| Carton adhesively sealed, dipped in wax, and wrapped with kraft paper | *A* | 0.085 | 0.141 | 0.161 | 0.233 |
| | *B* | 0.280 | 0.414 | 0.539 | 0.673 |
| | *C* | 0.168 | 0.350 | | |
| Carton adhesively sealed, dipped in wax, wax paper wrapped, heat-sealed, ends redipped, and the whole overwrapped with wax paper | *A* | 0.030 | 0.062 | 0.072 | 0.143 |
| | *B* | 0.095 | 0.215 | 0.234 | 0.352 |
| | *D* | 0.189 | 0.247 | | |
| Carton adhesively sealed, wrapped in heat-sealed wax-coated 300 M.S.T. Cellophane | *A* | 0.055 | 0.089 | 0.088 | 0.156 |
| | *A* | 0.159 | 0.283 | | |
| Carton pouch liner, heat-sealed, of wax-coated 300 M.S.T. Cellophane | *A* | 0.071 | 0.111 | 0.159 | 0.223 |
| | *A* | 0.185 | 0.241 | | |
| Rectangular fibre-coated composite can: metal ends, one with friction-plug lid; cardboard body; whole can dipped in wax after filling and closing | *C* | 0.141 | 0.245 | | |
| | *E* | 0.341 | 0.473 | | |

Inspection of data in Table II indicated wax-coated kraft as a likely alternative. Several waxes and methods of application, and two types of base stock were studied: the results shown in Table V indicated original kraft stock to be satisfactory, and that the most suitable coating of those studied was wax B (50% paraffin, 50% microcrystalline or amorphous wax) applied by a special double dip, i.e., a dip at high temperature to impregnate the board, followed by a dip at lower temperature to apply a surface coat. Under the test conditions employed there was no difference between sprayed and dipped packages.

Exposure and dropping tests were made on the completed rations (Table VI). The results verified the choice made from Table V, and further indicated that the spray treatment offered more protection to the contents when the packages were dropped at low temperatures. A kraft carton was designed on the basis of these experiments: this carton has been fully described elsewhere (2) and to date has been quite satisfactory.

TABLE V

WATER-VAPOUR PERMEABILITY OF WAXED KRAFT CARTONS 6¼ BY 4⅝ BY 3¾ IN. (139.4 SQ. IN.) CONTAINING CALCIUM CHLORIDE AND SUBJECTED TO 80° F. AND 85% RELATIVE HUMIDITY (VAPOUR PRESSURE DIFFERENCE, 22.4 MM. OF MERCURY)

| Stock material | Wax | Procedure | Water-vapour permeability, gm./wk. |
|---|---|---|---|
| Specially treated for wax dipping | *A* | Double dip | 0.186 |
| Specially treated for wax dipping | *B* | Double dip | 0.088 |
| Ordinary | *A* | Double dip | 0.179 |
| Ordinary | *B* | Double dip | 0.062** (0.018)* |
| Ordinary | *B* | Special double dip | 0.028** |
| Ordinary | *B* | Single dip | 1.288 |
| Ordinary | *B* | Single spray | 1.309 |
| Ordinary plus kraft wrap | *B* | Single spray | 0.635 |
| Ordinary kraft | *C* | Double dip | 0.070 (0.032)* |
| Ordinary kraft | *D* | Double dip | 0.534 |
| Ordinary kraft | *E* | Unknown | 7.4 |

* *Values after further tests using the small package with 50.7 sq. in. of transmitting surface.*
** *Best appearance after exposure to a temperature of −40° C.*

TABLE VI

WATER-VAPOUR PERMEABILITY OF COMPLETED ARMY MESS TIN RATION KITS SUBJECTED TO 80° F. AND 85% RELATIVE HUMIDITY (VAPOUR PRESSURE DIFFERENCE UNKNOWN)

| Type of container | Water-vapour permeability, gm./wk. | | |
|---|---|---|---|
| | As received | After exposure to −40° C. | After dropping four times at −40° C. |
| Old kit* | 0.381 | 0.459 | 1.904 |
| New kit, kraft wrapped followed by double wax dip | 0.431 | — | 2.331 |
| New kit, special double wax dip | 0.497 | 0.438 | 1.039 |
| New kit, double wax spray corresponding to special double wax dip | 0.435 | 0.560 | 0.560 |

* *Smaller than new kit.*

## Acknowledgments

The authors wish to express their gratitude to the many commercial firms, both American and Canadian, who so kindly contributed materials and advice during the course of this work; and to Mr. H. Tessier of these laboratories for his technical assistance.

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# PACKAGING

## II. A CELLULOSE-BASE CONTAINER FOR MODIFIED VACUUM PACKING[1]

By A. H. Woodcock[2]


### Abstract

Films of wax-coated laminated "Cellophane" have been shown to transmit carbon dioxide 25 times as rapidly as they transmit oxygen. Packages made from this film and gas-packed with carbon dioxide produce a vacuum pack on standing. Factory trials indicated that this type of packaging is feasible commercially, and shipping trials have shown the package to be reasonably substantial. Storage trials at 26.7° and 37.8° C. showed the package to be effective for a period of six months.


### Introduction

The need for non-metallic food containers having low water-vapour permeability has been discussed in an earlier publication (10). Valuable constituents of many foodstuffs, carotene (4), ascorbic acid (1), butter fat (5), and other fats, are believed to be susceptible to deterioration in the presence of oxygen and to require protection from it. Complete protection can be obtained only by the use of packages made of material impermeable to oxygen and hermetically sealed. For this purpose, tin plate is one of the most suitable materials. The present shortages of materials and cost considerations have focused attention on non-metallic films with low oxygen permeability.

While the previous paper in this series was confined to a study of the effectiveness of non-metallic containers as water-vapour barriers, it was possible to utilize some of this information in the development of a package having both low water-vapour permeability and low oxygen permeability.

The present paper describes a non-metallic food container with low oxygen permeability believed suitable for packaging dried whole milk powders and other powdered products subject to oxidative deterioration.

### Experimental

The apparatus used to measure the oxygen permeability of these films was similar to one described elsewhere (6) and is shown in Fig. 1. The film was placed between two hemispherical cups *A* and *B*. Both sides of the film were evacuated and the cup *B* was filled with gas at a few centimetres' pressure as measured by the manometer *C*. The vacuum pump was shut off from *A* by raising the mercury level above the point *D*, and gas diffused into *A* from *B* through the film. At regular periods the amount of gas in *A* was determined by raising the mercury level and compressing it into the calibrated

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[2] *Formerly Biophysicist, Food Investigations; now Research Chemist, E.S. and A. Robinson (Canada) Ltd.*

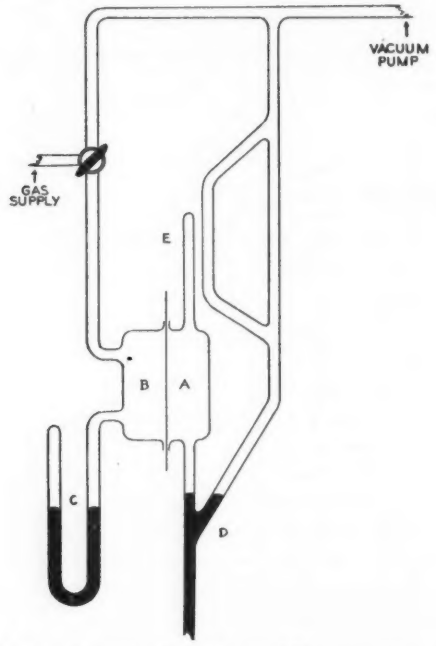


FIG. 1. *Apparatus for gas permeability measurements*

tube *E*, where its volume and pressure were measured in a manner similar to that used with a McLeod gauge. Compensation for pressure of volatile vapours, which reached saturation under compression, was made by taking a series of readings at different volumes. Measurements at 24-hr. intervals permitted calculation of the permeability in absolute units.

Following development of a film with low oxygen permeability, packages were fabricated, filled with milk powder, sealed, and gas-packed. Some of these were subjected to shipping trials, some to rough treatment at low temperatures, and others were put into storage at 26.7° and 37.8° C. (80° and 100° F.).

The storage experiment was designed to compare the quality of dried whole milk powder packed in air and packed in inert atmospheres using the current commercial method and these non-metallic containers. One-pound tins were used for the commercial pack while 8 oz. and 20 lb. packages were used for the non-metallic gas-packs. Milk powder quality was determined at three-month intervals by tasting tests (7).

## Results

### *Description of Film Developed*

After several unsuccessful trials a film with low oxygen permeability was designed. This film consisted of two layers of 450 M.S.Y.T. "Cellophane"

laminated together with lacquer and coated on one side with a flexible wax compound (40 lb. per ream of 500 sheets 24 by 36 in.). The water-vapour permeability of this film when formed into a liner was found to be about one-eighth of that of the film used for the dried egg package (10).

*Oxygen and Carbon Dioxide and Permeability of the Film*

The oxygen and carbon dioxide permeability of this film as prepared is shown in Table I. After tests had been repeated several times on a sample of film, the permeability appeared to decrease; this change was believed to be due to the partial dehydration of the film when subjected to repeated evacuation. Drying a sample by subjecting it to vacuum at room temperature confirmed this (Table I). Furthermore, the ratio of permeability to oxygen

TABLE I

OXYGEN AND CARBON DIOXIDE PERMEABILITY OF LAMINATED 450 M.S.Y.T. CELLOPHANE, COATED ON ONE SIDE WITH A FLEXIBLE WAX COMPOUND

| Condition of film | Penetration in ml. per sq. metre per 24 hr. per mm. pressure difference | | |
|---|---|---|---|
| | Oxygen | Carbon dioxide | Ratio |
| As received | 0.0075 | 0.186 | 24.8 |
| Dried under vacuum | 0.0036 | 0.091 | 25.2 |

and carbon dioxide was proportional, within the limits of experimental error, to the ratio of their solubilities in water at room temperature, i.e. 1 : 27, indicating that the Cellophane, which contains moisture, is the effective part of the film rather than the heat-sealing lacquer, laminating compound, or the wax coating. This had been observed previously for regenerated cellulose films (9); however, for films such as Pliofilm the ratio of permeabilities is believed to be about 1 : 4 (2).

Based on the values reported in Table I, a cubic package with 6 in. sides would transmit 60 ml. of oxygen per annum. Since this package should contain about 2500 gm. of milk powder, the amount of oxygen transmitted would be below the critical level associated with rapid fat deterioration in milk powders (5), if uniformly distributed through the contents. Should it react with the surface layer of milk, rancidity might occur, and the storage investigation was planned to compare the effectiveness of this method of gas-packing milk powders with the method currently in industrial use.

*Packages of Low Oxygen Permeability*

When a package prepared from this film is filled with carbon dioxide the partial pressure differential across the film is approximately 758 mm. of mercury since the partial pressure of carbon dioxide under normal atmospheric conditions is 2.28 mm. of mercury. It follows from Dalton's law that the

carbon dioxide will be lost from the package more rapidly than it can enter (3), and, since the diffusion rate of oxygen (Table I) in the reverse direction is much less, a partial vacuum is created. This was demonstrated by forming a pouch-type liner bag of this wax-coated laminated material over a paperboard frame and sealing. The packages were filled with carbon dioxide through a small hole (brogue hole), by evacuating in a chamber and replacing the air with carbon dioxide; the brogue hole was sealed with a drop of melted wax. On standing, carbon dioxide escaped and the bag collapsed as shown in Fig. 2. Calculations based on the results of Table I indicate that carbon dioxide would be lost at an initial rate of 14.1 cu. mm. per sq. cm. per day.

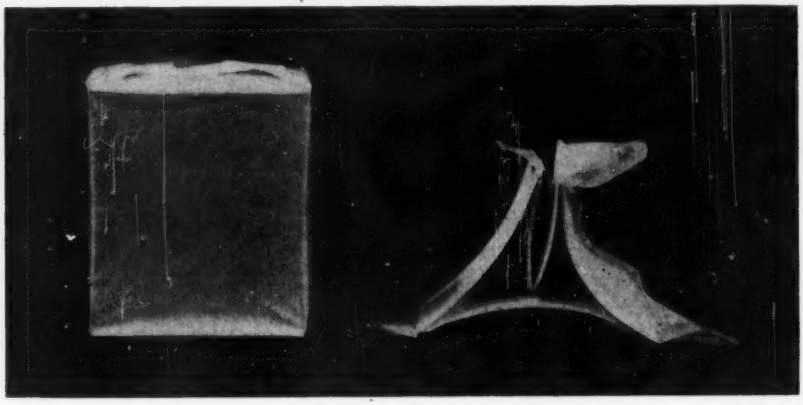

FIG. 2. *Comparison of package freshly filled with carbon dioxide and package after standing several days.*

A foodstuff packed in this manner was essentially vacuum-packed, since the carbon dioxide diffused in the atmosphere or was sorbed by the milk powder (8). Successful packs were therefore compressed into a firm block by atmospheric pressure and remained firm as long as the vacuum was maintained. A damaged package became soft again; this provided a simple test for faulty packs.

*Tests*

Dropping tests using packages containing 8 oz. of milk powder (30 in. on to a cement floor) were conducted at low temperatures. Two packages dropped at −40° C. (−40° F.) were fractured, one package out of two fractured at −15.6° C. (+ 4° F.), while both of two packages remained gas-tight at −22° C. (28° F.), indicating that at sharp freezing temperatures the package became too brittle to withstand rough handling.

A factory test was made with 50 packages of this type, each packed with 20 lb. of dried whole milk powder. The only extra equipment required was a mandrel and sealing irons. One package only was defective; this indicated reasonable commercial feasibility. Shipping tests were done with four of these

packages packed in a corrugated master carton. Packages were shipped by express, examined, and returned, then immediately reshipped by freight. On the return journey by freight one package broke down.

The results of the storage experiment are shown in Fig. 3; the experiment was concluded after nine months of storage since many of the flexible con-

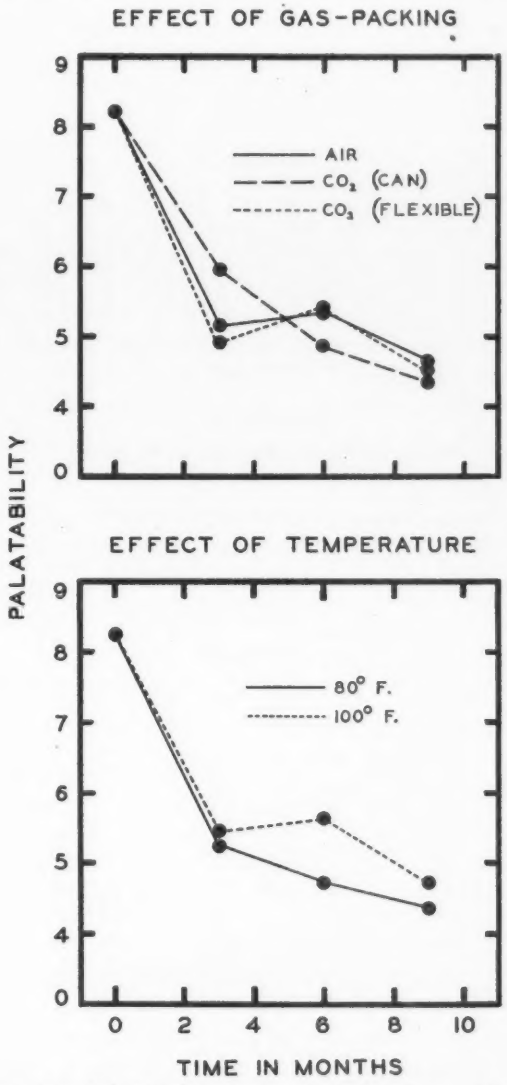


FIG. 3. *Effects of method of packing (average for both temperatures) and temperature (average for all methods of packaging) on the palatability of stored milk powder.*

tainers had lost their vacuum. Gas-packing by either method appeared to effect little improvement in the quality of stored milk powders. The better palatability in samples stored at temperatures of about 37.8° C. has been noted (7); both of these features are under further investigation (8).

Flexible packs were tested at each sampling time by their hardness. At six-month sampling all the non-metallic containers were still hard. At the nine-month sampling, all 8 oz. and 20 lb. containers at 37.8° C. were soft, while at 26.7° C. one of three 8 oz. and one of three 20 lb. containers were still hard. Alterations to extend the usefulness of this pack beyond this six-month limit are under investigation.

## Acknowledgments

The author wishes to express his thanks to E. S. and A. Robinson (Canada) Ltd., who kindly prepared the films, examined the packages used in this investigation and assisted in shipping trials; to Cow and Gate (Canada) Ltd., at whose factory commercial trials were made; to Dr. J. A. Pearce, Biochemist, National Research Laboratories, who conducted the storage experiments; and to Mr. H. Tessier, Laboratory Assistant, National Research Laboratories, Ottawa, Canada, for his technical assistance.

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# RATION BISCUITS

## II. EFFECT OF TYPE AND CONCENTRATION OF SHORTENING ON KEEPING QUALITY[1]

By G. A. Grant[2], J. B. Marshall[3], and W. Harold White[4]


## Abstract

Ration biscuits prepared by two manufacturers and containing 8 to 23% of one compound animal–vegetable and three all vegetable shortenings were stored at 43.3° C. (110° F.) for 36 wk. Keeping quality was assessed by flavour, peroxide oxygen, and pH determinations.

The type of shortening was found to have a greater effect on keeping quality than the fat concentration or plant practice. Biscuits made with stabilized hydrogenated vegetable shortening were more stable than biscuits made with a compound animal–vegetable shortening. All biscuits became objectionable to the tasters after storage for 22 wk.


## Introduction

Interest in the keeping quality and nutritional properties of ration biscuits has been stimulated by requirements of the armed services (6). The desirability of high fat biscuits as a means of increasing the caloric value of a ration must be considered in relation to the vulnerability of the shortening to oxidative decomposition when combined with other ingredients in a baked product (2). As far as can be ascertained there is little information available on the effect of concentration of shortening on the keeping quality of biscuits or crackers. This paper describes the results of an experiment in which the levels of four shortenings were varied in a simple formula, to determine the effect of the amount of each fat on its stability in a baked product.

## Materials

The experimental material consisted of two lots of biscuits prepared independently by commercial manufacturers, using similar ingredients in a simple formula. Varying amounts of four shortenings drawn from stocks available in the biscuit plants were mixed with 50 lb. of soft wheat flour, from a common source, water added according to bakeshop practice, and soda used for leavening. Details of the proportions used are shown in Table I. The amounts of shortening used were calculated to give fat levels of 8, 13, 18, and 23% in the final product; reference to Table II shows that most of the fat was retained in the biscuits even at the highest levels.

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TABLE I

BAKING FORMULAE OF BISCUITS MADE WITH FOUR SHORTENING LEVELS

| Ingredients | Shortening levels | | | |
|---|---|---|---|---|
| | 8% | 13% | 18% | 23% |
| Shortening | 4 lb. 6 oz. | 7 lb. 8 oz. | 11 lb. | 14 lb. ½ oz. |
| Soft wheat flour | 50 lb. | 50 lb. | 50 lb. | 50 lb. |
| Water | 17 lb. | 15 lb. | 13 lb. 8 oz. | 11 lb. 8 oz. |
| Baking soda— | | | | |
| Plant A | 6 oz. | 6 oz. | 6 oz. | 6 oz. |
| Plant B | 8 oz. | 8 oz. | 8 oz. | 8 oz. |

TABLE II

MOISTURE AND FAT CONTENT OF BISCUITS MADE WITH FOUR TYPES AND LEVELS OF SHORTENING IN TWO PLANTS

| Shortening | Calculated shortening level, % | Fat and moisture content as determined experimentally | | | |
|---|---|---|---|---|---|
| | | Plant *A* | | Plant *B* | |
| | | Fat, % | Moisture, % | Fat, % | Moisture, % |
| 1 | 8 | 7.5 | 8.3 | 7.9 | 8.4 |
| | 13 | 13.3 | 7.5 | 13.0 | 7.2 |
| | 18 | 18.9 | 5.5 | 18.3 | 6.0 |
| | 23 | 23.3 | 5.1 | 21.0 | 5.3 |
| 2 | 8 | 8·4 | 8.0 | 8.1 | 8.8 |
| | 13 | 13.0 | 7.2 | 13.3 | 6.8 |
| | 18 | 19.6 | 5.7 | 17.7 | 5.9 |
| | 23 | 22.8 | 5.0 | 21.0 | 5.7 |
| 3 | 8 | 7.6 | 8.4 | 9.9 | 6.9 |
| | 13 | 12.8 | 7.2 | 15.6 | 6.1 |
| | 18 | 19.0 | 6.0 | 20.0 | 5.4 |
| | 23 | 21.7 | 5.3 | 22.9 | 5.8 |
| 4 | 8 | 7.5 | 8.2 | 7.6 | 7.7 |
| | 13 | 12.0 | 6.3 | 12.9 | 6.7 |
| | 18 | 18.2 | 5.4 | 18.3 | 5.6 |
| | 23 | 22.5 | 4.5 | 21.2 | 5.6 |

The shortenings were from commercial stocks available at the time the experiment was started, and included one compound animal–vegetable and three hydrogenated and stabilized vegetable products. Some analytical characteristics of samples drawn from the materials used are given in Table III.

## Methods

The biscuits were stored at 43.3° C. (110° F.) in fibreboard containers lined with thin glazed paper, each box holding about 20 lb. The containers were overwrapped with waxed paper and finally with brown paper, these wrappings being carefully replaced after each sampling.

TABLE III

CHARACTERISTICS OF SHORTENINGS USED IN EXPERIMENTAL BISCUITS

| Type of shortening | Swift stability time at 110° C. (hr.) | Characteristics | | | | |
|---|---|---|---|---|---|---|
| | | Saponification No. | Iodine No. | Smoke point, °C. | Refractive index at 48° C. | Capillary melting point, °C. |
| 1. Compound animal–vegetable | 15 | 188.3 | 69.0 | 221 | 1.4580 | 49.0 |
| 2. Stabilized hydrogenated all-vegetable | 20 | 188.6 | 67.3 | 213 | 1.4589 | 37.3 |
| 3. Stabilized hydrogenated all-vegetable | 89 | 187.5 | 52.2 | 218 | 1.4565 | 40.3 |
| 4. Stabilized hydrogenated all-vegetable | 43 | 191.3 | 62.9 | 215 | 1.4579 | 41.9 |

Quality was assessed on the whole biscuits by 16 tasters, who were required to score six samples in the morning and six in the afternoon on the following basis:— 10, excellent, fresh flavour and odour; 8, good, no off-flavour or odour; 6, fair, slight off-flavour or odour; 4, poor, marked off-flavour and odour; 2, very poor, offensive odour and flavour; and 0, inedible.

Peroxide values were determined by a method commonly used in these laboratories (7). The biscuits were ground with a rolling pin and fat extracted as follows:— Twenty grams of material was placed in a 100 ml. centrifuge tube, and 40 to 50 ml. of petrol ether (b.p. 30° to 50° C.) added; the mixture was thoroughly stirred with a power mixer and the extract decanted through a No. 4 Whatman filter paper into a 125 ml. Erlenmeyer flask. This procedure was carried out three times. Most of the petrol ether was then removed on a boiling water-bath and final traces by placing the flasks in a vacuum oven for about 30 min. at 40° C.

Preliminary experiments showed that sufficient fat for the analyses could be obtained by three extractions and that no appreciable difference in the peroxide oxygen values obtained resulted from increasing the number of extractions.

Measurements of pH were made on potassium chloride extracts of the ground biscuit material, using a 20 to 1 ratio of a 10% salt solution. The samples were weighed, salt solution added, and stirred four or five times before the supernatant solution was decanted for measurement of the hydrogen ion concentration. This procedure was rapid and facilitated handling large numbers of samples quickly and gave results that were comparable with those from more elaborate extraction methods.

The iodine numbers of the shortenings were determined by Kaufmann method (1, p. 432) and the saponification numbers by the official A.O.A.C. method (3).

## Results

*Flavour*

Changes in mean flavour scores are shown in Fig. 1. While somewhat irregular, the general trends were similar throughout, and suggest that the highest concentrations of fat had a greater effect on the reaction of the tasters to flavour deterioration. There was also a difference between plants with respect to the flavour scores assigned to the biscuits of the same fat level. As was to be expected, the mixed animal–vegetable shortening showed the most rapid change.

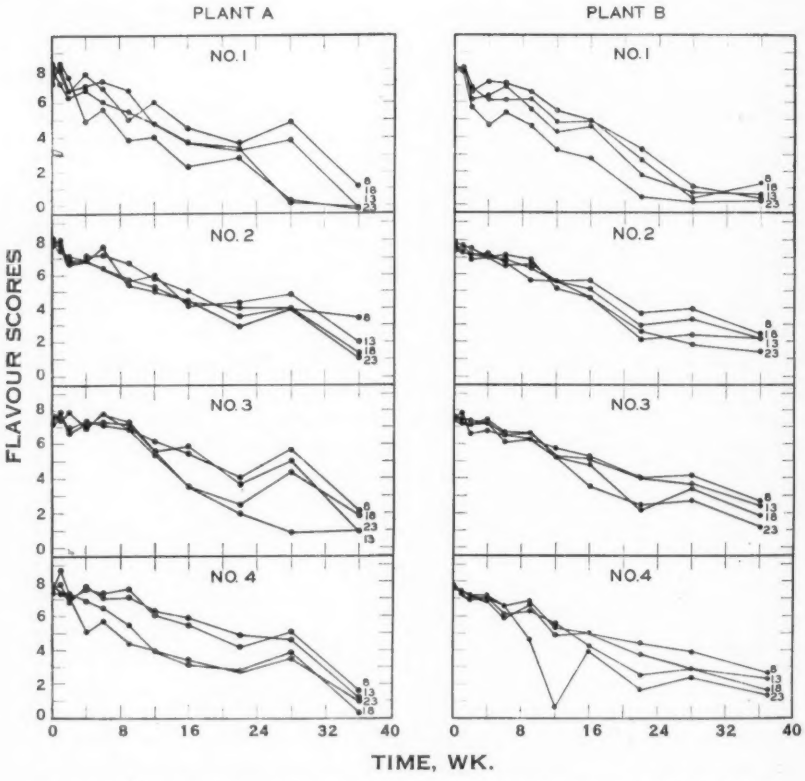


FIG. 1. *Effect of shortening concentration 8 to 23%, on flavour scores of ration biscuits stored at 43.3° C. (110° F.).*

*Peroxide Oxygen*

Increases in the peroxide oxygen values are shown in Fig. 2 for all of the variables tested. Differences between shortenings were more pronounced than the effects of amount of shortening or plant practice.

The shortest induction periods and greatest peroxide oxygen values were found in the material made from the compound animal–vegetable shortening, and the greatest stability with the hydrogenated and stabilized product, No. 3. Samples 2 and 4 gave indication of having reached the ends of their induction periods in the material from Plant A.

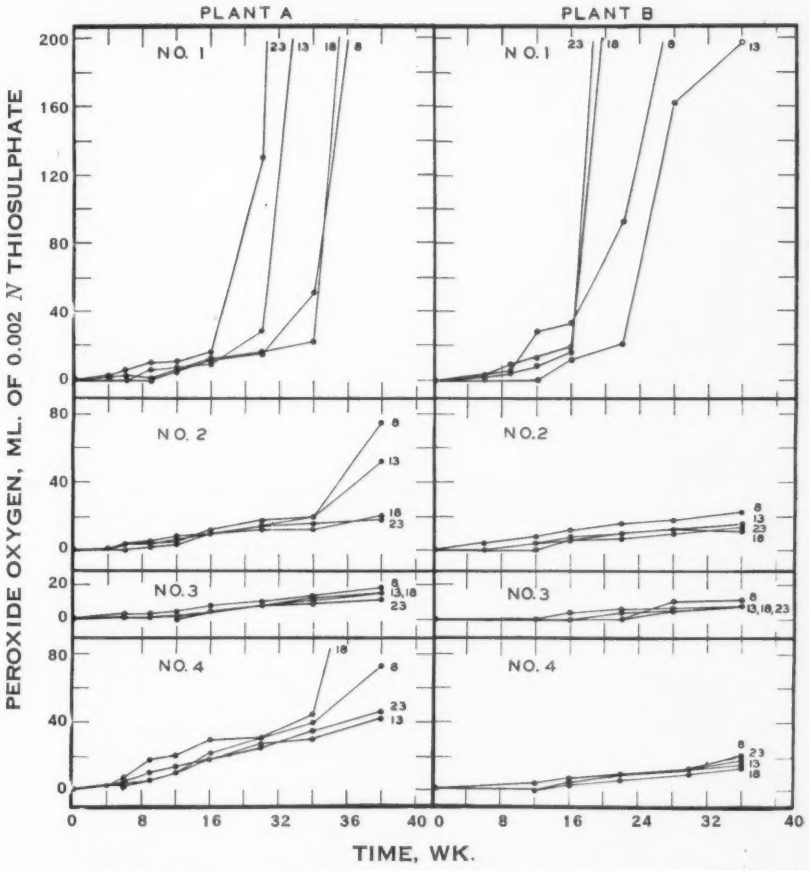


FIG. 2. *Effect of shortening concentration 8 to 23% on peroxide oxygen development in ration biscuits stored at 43.3° C. (110° F.)*

*pH*

The initial reactions of the test materials were quite alkaline and the changes shown in Fig. 3 gradual and somewhat erratic. No appreciable effect of shortening concentration was evident and only a slight indication of more rapid changes in the products from Plant A. The general trend was similar to that for flavour scores.

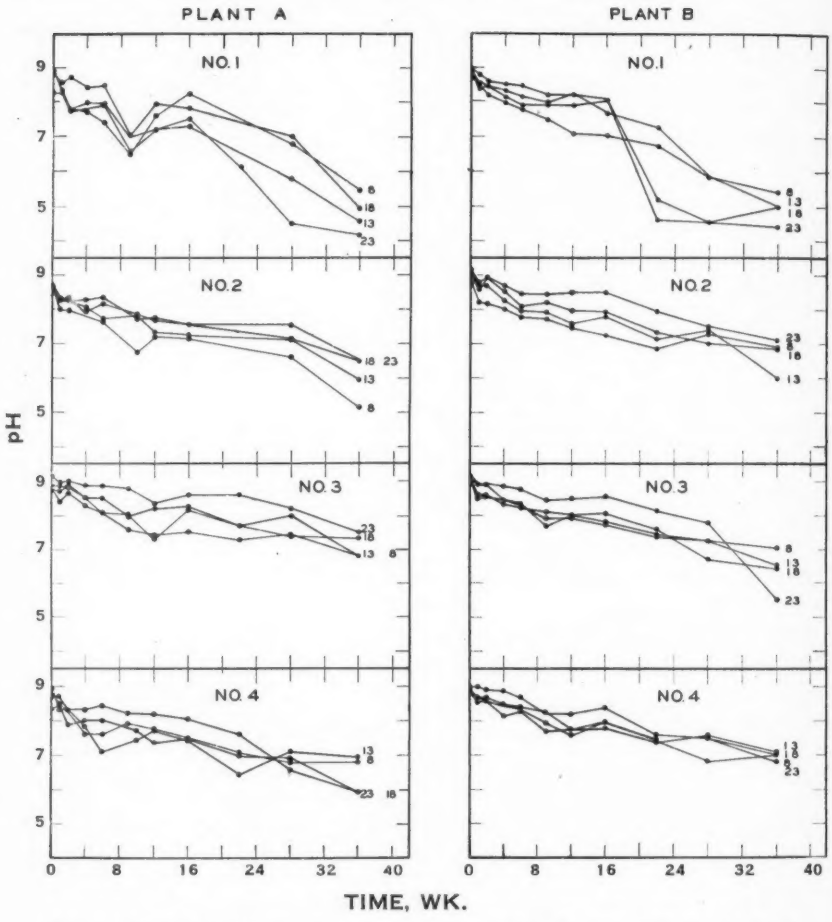


FIG. 3. *Effect of shortening concentration 8 to 23% on pH values of ration biscuits stored at 43.3° C. (110° F.).*

Gradual increases in the acidity of stored flour and wheat germ have been observed (4, 5). Since the changes for material made with the mixed animal–vegetable shortening were greater and more rapid than those for the hydrogenated shortenings, the pH measurements appear to have reflected as well a change in the shortening that corresponds roughly to the end of the induction period.

*Mean Values*

Mean values for peroxide oxygen, flavour score, and pH are shown in Table IV, the data being averaged for the variables as indicated.

TABLE IV

THE EFFECT OF SHORTENING LEVEL, TYPE OF SHORTENING, PLANT PRACTICE, AND TIME OF STORAGE ON THE DEVELOPMENT OF MEAN PEROXIDE OXYGEN, FLAVOUR, AND pH

| Shortening level | 8.0% | 13.0% | 18% | 23.0% |
|---|---|---|---|---|
| Mean peroxide oxygen[1] | 16.0 | 16.5 | 20.3 | 24.6 |
| Mean flavour[1] | 5.7 | 5.6 | 5.3 | 4.8 |
| Mean pH[1] | 7.3 | 7.5 | 7.7 | 7.8 |
| **Shortening** | **1** | **2** | **3** | **4** |
| Mean peroxide oxygen[1] | 56.0 | 11.6 | 3.1 | 6.7 |
| Mean flavour[1] | 4.8 | 5.4 | 5.6 | 5.6 |
| Mean pH[1] | 6.9 | 7.8 | 8.1 | 7.2 |
| **Plant** | ***A*** | | ***B*** | |
| Mean peroxide oxygen[1] | 22.9 | | 16.9 | |
| Mean flavour[1] | 5.5 | | 5.2 | |
| Mean pH[1] | 7.3 | | 7.8 | |

| Time in weeks | 0 | 1 | 2 | 4 | 6 | 9 | 12 | 16 | 22 | 28 | 36 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean peroxide oxygen[1] | 0 | 1 | 1 | 1 | 2 | 3 | 6 | 11 | 42 | 71 | 76 |
| Mean flavour[1] | 7.7 | 7.8 | 6.9 | 6.8 | 6.5 | 6.11 | 5.1 | 4.4 | 3.1 | 3.1 | 1.5 |
| Mean pH[1] | 8.9 | 8.5 | 8.2 | 8.0 | 8.1 | 7.8 | 7.8 | 7.8 | 7.2 | 6.9 | 6.1 |

[1] *Mean value over all other conditions studied for biscuit stored at 43.3° C. (110°F.)*

The differences resulting from the shortening levels were relatively small for all the measurements. The 23% level had the highest mean peroxide oxygen and lowest flavour scores. The apparently greater decrease in pH values at the lower shortening levels was due to the interaction of the levels with time and with shortenings.

There was little to choose among the shortenings except for shortening No. 1 (animal–vegetable), which yielded a biscuit with lower mean flavour and mean pH values and a much higher mean peroxide oxygen value than the other samples. The stability of the biscuits prepared with this shortening appeared to be markedly inferior to the others.

The magnitude of the differences occurring between plants was small. Plant *B* had lower mean peroxide oxygen and flavour and higher mean pH values.

An increase in mean peroxide oxygen content and a decrease in mean flavour and pH values occurred with time of storage. The change in mean peroxide oxygen value was greatest between storage for 22 and 36 wk., while the

largest change in mean flavour scores was evident between 9 and 36 wk. The changes in mean pH were gradual, with the greatest decrease resulting for shortening No. 1.

*Analyses of Variance*

The significance of the effects of the factors investigated was tested statistically by means of analyses of variance. Since the compound animal–vegetable shortening (No. 1) behaved quite differently from the others, the data pertaining to it were omitted from the computations with respect to peroxide oxygen. Moreover, as the changes in flavour score and peroxide oxygen values were small during the first stages of the experiment, only data for the period between 9 and 36 wk. were included in the statistical analyses.

Analyses of variance for flavour scores and peroxide oxygen values are given in Table V and for the pH data in Table VI. Differences among shortenings Nos. 2, 3, and 4 with respect to flavour were not significant and shortening level did not affect the peroxide oxygen values or the pH measurements significantly.

TABLE V

ANALYSES OF VARIANCE OF FLAVOUR AND PEROXIDE OXYGEN DATA FOR RATION BISCUITS STORED AT 43.3° C. (110° F.)

| Source of variance | Degrees of freedom | Flavour value, mean square | Degrees of freedom | Peroxide oxygen value, mean square |
|---|---|---|---|---|
| Shortenings | 3 | 12.58** | 2 | 2726** |
| No. 1 vs. others | 1 | 35.43** | | |
| Others | 2 | 0.57 | | |
| Shortening levels | 3 | 19.26** | 3 | 237 |
| Linear regression on shortening levels | 1 | 55.54** | | |
| Time | 5 | 88.68** | 5 | 2537** |
| Plants | 1 | 3.47** | 1 | 5277** |
| Plants × time | 5 | 3.34** | 5 | 729** |
| Plants × shortening level | 3 | 0.08 | 3 | 236 |
| Plants × shortenings | 3 | 0.15 | 2 | 1797** |
| Shortening level × shortenings | 9 | 1.12* | 6 | 272 |
| Shortening level × time | 15 | 0.54 | 15 | 122 |
| Shortening × time | 15 | 1.11** | 10 | 373* |
| Time × No. 1 vs. others | 5 | 2.09** | | |
| Others | 10 | 0.61 | | |
| Residual | 129 | 0.48 | 91 | 177 |

* Indicates 5% level of statistical significance.
** Indicates 1% level of statistical significance.

## Discussion

Increasing the fat concentration in ration biscuits did not significantly affect their stability as assessed by peroxide oxygen determinations and pH measurements, but as judged by flavour score the higher levels gave somewhat less acceptable products after storage at 43.3° C. (110° F.). Peroxides developed most rapidly and reached the highest concentration in the biscuits made with compound animal–vegetable shortening and this material also attained

TABLE VI

ANALYSIS OF VARIANCE OF pH DATA FOR RATION BISCUITS STORED AT 43.3° C. (110° F.)

| Source of variance | Degrees of freedom | Mean square |
|---|---|---|
| Shortening levels | 3 | 0.874 |
| Shortenings | 3 | |
| No. 1 vs. others | 1 | 17.590* |
| Others | 2 | 1.654 |
| Time | 7 | 24.657** |
| Plants | 1 | 2.655** |
| Plants × shortening levels | 3 | 0.044 |
| Plants × shortenings | 3 | 0.890* |
| Plants × time | 7 | 0.238 |
| Time × shortenings | 21 | |
| No. 1 vs. others × time | 7 | 3.024** |
| Others × time | 14 | 0.051 |
| Shortening levels × shortenings | 9 | |
| 23% vs. others × shortenings | 3 | 1.582** |
| Others × shortenings | 6 | 0.2374* |
| Plants × time × shortenings | 21 | 0.2351** |
| Residual | 156 | 0.0954 |

** Indicates 1% level of statistical significance.
* Indicates 5% level of statistical significance.

the lowest rating in the organoleptic tests. All of the material had become objectionable after storage for 22 wk., off flavours and odours being detected before significant changes took place in some of the fats. Presumably this may have resulted from partial deterioration of the non-fat fraction. Variations in behaviour between plants may have been due in part to the difference in the amounts of baking soda used. This factor has been investigated in greater detail in these laboratories*.

On the basis of the present investigation, it is concluded that if a ration biscuit of high caloric value is required, fat levels could be safely increased by using stable shortenings without significantly altering the keeping quality of the biscuit.

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# VARIETAL DIFFERENCES IN BARLEYS AND MALTS

## XIV. INTERVARIETAL RELATIONS BETWEEN WORT PROPERTIES AND BARLEY AND MALT PROPERTIES[1]

By W. O. S. Meredith[2] and H. R. Sallans[3]


### Abstract

Data representing 24 barley varieties grown at six experimental stations in Canada were used to examine intervarietal relations among wort properties (degree of attenuation, viscosity, initial turbidity, final turbidity, and stability) and a number of barley, malting, and malt properties.

The wort properties show significant associations with malt extract, saccharifying activity (Lintner value), and wort nitrogen, and also with barley salt-soluble nitrogen, hours steep, and malting loss, but they are not significantly related to barley starch, extract, or Lintner value after activation with papain. Degree of attenuation and stability increase, while viscosity and turbidity decrease, with increases in malt extract, saccharifying activity, wort nitrogen, barley salt-soluble nitrogen, and malting loss. It is concluded that the wort qualities are dependent on the development of enzymes in the growing barley and hence they reflect the extent of malt modification.

Only one of the correlation coefficients is of such magnitude that a single malt property can be regarded as a measure of a wort property. This is the coefficient ($r = .842$) between wort nitrogen and wort viscosity. The other associations discussed, though significant, are loose, and it is concluded that wort properties cannot be adequately predicted from the commonly measured barley and malt properties.

It is suggested that the results of quality tests on laboratory worts give information of value in assessing the quality of brewery worts.


The studies reported in the preceding 13 papers in this series were undertaken to elucidate varietal differences in a large number of barley, malting, and malt properties. Associations between the properties were also examined in an effort to throw some light on the nature of malting quality in barley (3). Certain of the associations have been put to practical use by the development of prediction methods (5, 10) whereby estimates of malt extract and saccharogenic activity (Lintner value) are obtained from small samples of barley. As a result, it is possible to provide information on the malting quality of new breeding lines at an early stage in their production; the plant breeder is able to concentrate on the most promising lines; and the testing capacity of the malting laboratory is increased because the number of samples subjected to the time-consuming and expensive malting test is reduced.

Wort quality determinations have been studied and were described in Part XIII (9) of this series. It is therefore logical to examine the relations

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between the quantity and quality of malt extract, and between the enzymatic properties—represented by Lintner value and wort nitrogen content—and wort properties. Further, as malt extract and Lintner value have been shown to be closely related to barley properties (3, 11, 12, 13), it is also logical to examine the relations between wort qualities and barley starch, extract, Lintner value after activation by papain, salt-soluble nitrogen content, steeping time, and malting loss.

The relations between wort qualities and barley and malt properties for the 24 varieties described in Part XIII (9) are discussed in this paper. These relations are examined to determine the extent to which wort properties are related to other properties with the view of offering some explanation for varietal differences in wort quality and of determining whether wort qualities can be predicted from barley or malt properties.

## Data and Methods

The experimental data were obtained from 144 samples of barley and the malts made from them. The samples represented 24 varieties grown at six widely scattered experimental stations in Canada. A description of the varieties was published in Part XIII of this series (9). The methods used in growing and harvesting the samples were described in Part I (1), and the malting methods were described in Part IV (6). The data for degree of wort attenuation, viscosity, and turbidity were presented in Part XIII (9). Barley extract, starch, salt-soluble nitrogen content, and Lintner value after activation by papain were determined in the National Research Laboratories by the methods previously described (1, 4, 11). The results of these tests have been discussed elsewhere in dealing with varietal characteristics (2, 9), but for convenience the data on the various barley, malting, and malt properties are summarized in Appendix Tables I and II.

The relations between wort properties and barley and malt properties were examined by means of correlation studies. Only intervarietal relations, that is, those between varietal means, are reported. The small number of stations included in the study is insufficient for the study of intravarietal relations.

## Correlation Coefficients

The simple intervarietal correlation coefficients between wort properties and barley and malt properties are given in Table I. As certain of the coefficients between the various barley and malt properties are of value in explaining some of the factors contributing to wort quality, simple correlation coefficients between the malt and barley properties are given in Table II. The correlation coefficients among the wort properties have been presented in a previous paper (9).

Of the 55 correlation coefficients involving wort qualities (Table I) 13 exceed the 1% level of significance, 10 exceed the 5% level, 5 just fail to attain the 5% level, and 27 are not significant.

TABLE I

SIMPLE INTERVARIETAL CORRELATION COEFFICIENTS BETWEEN WORT PROPERTIES AND BARLEY, MALTING, AND MALT PROPERTIES

| Property | Wort properties | | | | |
|---|---|---|---|---|---|
| | Attenuation, % | Viscosity | Initial turbidity, % | Final turbidity, % | Stability |
| Barley | | | | | |
| Total nitrogen, % | −.072 | −.132 | .112 | .057 | −.008 |
| Salt-soluble nitrogen, % | .424* | .671** | −.316 | −.424* | .439* |
| 1000-kernel wt., gm. | −.304 | .489* | .064 | .150 | −.187 |
| Starch, % | .329 | −.103 | −.080 | −.164 | .200 |
| Extract, % | .334 | −.108 | −.131 | −.214 | .236 |
| Saccharogenic activ. °L. | .251 | .042 | .050 | .088 | −.100 |
| Malting | | | | | |
| Hours steep | −.391 | .382 | .534** | .559** | −.492* |
| Malting loss, % | .370 | −.810** | −.439* | −.550** | .543** |
| Malt | | | | | |
| Extract, % | .572** | −.559** | −.363 | −.487* | .503* |
| Saccharogenic activ., °L. | .550** | −.520** | −.375 | −.398 | .355 |
| Wort nitrogen, % | .461* | −.842** | −.438* | −.594** | .614** |

NOTE: *In this table and Table II * denotes that the 5%, and ** that the 1%, level of significance has been attained.*

TABLE II

SIMPLE INTERVARIETAL CORRELATION COEFFICIENTS BETWEEN BARLEY, MALTING, AND MALT PROPERTIES

| Property | Malt properties | | |
|---|---|---|---|
| | Extract, % | Saccharogenic activity, °L. | Wort nitrogen, % |
| Barley | | | |
| Total nitrogen, % | −.282 | .448* | .517** |
| Salt-soluble nitrogen, % | .496* | .690** | .554** |
| 1000-kernel wt., gm. | −.211 | −.211 | −.394 |
| Starch, % | .785** | −.227 | −.163 |
| Extract, % | .854** | −.098 | −.042 |
| Saccharogenic activity, °L. | −.088 | .753** | −.274 |
| Malting | | | |
| Hours steep | −.485* | −.433* | −.519** |
| Malting loss, % | .693** | .388 | .736** |
| Malt | | | |
| Extract, % | — | .260 | .395 |
| Saccharogenic activity, °L. | .260 | — | .743** |
| Wort nitrogen, % | .395 | .743** | — |

Of the coefficients that are not significant, 24 out of the 27 are between wort qualities and the barley properties: nitrogen, 1000-kernel weight, starch, extract, and $\beta$-amylase activity (Lintner value). The significant correlations are those between wort qualities and barley salt-soluble nitrogen, hours steep, malting loss, malt extract, malt saccharogenic activity, and wort

nitrogen content. Thus it appears that wort quality is related to the amounts of extract and enzymes *actually developed* during malting and *not* to the amounts of extract or $\beta$-amylase that are *potentially available* in the barley. In other words, wort qualities reflect the extent to which the barleys are modified in the production of malt. This point will be discussed in greater detail after an examination of the relations involving each wort property.

Wort attenuation is significantly and directly related to barley salt-soluble nitrogen, malt extract, malt saccharogenic activity, and wort nitrogen content, and the correlation coefficients for steeping time and malting loss with degree of attenuation approach the 5% level of significance. Since salt-soluble nitrogen, steeping time, and malting loss are associated with malt extract, malt saccharogenic activity, and wort nitrogen content (see Table II), it is evident that all these properties influence or reflect enzymatic development which is in turn reflected by the quantity of fermentable material in wort as measured by degree of attenuation. Among these complex factors, malt extract and malt saccharogenic activity appear to be the most important single factors in determining degree of wort attenuation.

Wort viscosity exhibits relations similar to those for degree of attenuation, and the same properties are involved. In general the correlation coefficients are somewhat higher than those for degree of attenuation. This may be explained on the basis that wort viscosity measures less complex factors than does fermentability. The relation involving 1000-kernel weight with fermentability is not significant, but it is significant with viscosity. The same conclusions as were drawn in the previous section with respect to the effect of modification of the barley on wort quality apply to the relations involving wort viscosity. In fact, additional confirmatory evidence is obtained from the significant direct correlation between kernel size and viscosity. It is well established that small kernels modify more readily than larger kernels, and these data indicate that with increase in kernel size there is an accompanying increase in wort viscosity. Hence there is further reason for associating wort viscosity with extent of malt modification. Among the factors contributing to wort viscosity, malt extract and wort nitrogen content appear to be the most important.

Initial turbidity, final turbidity, and stability are very similar in their relations with the other properties, and these relations resemble those for degree of attenuation and wort viscosity. There are no outstanding features about the relations, except that they provide additional evidence that wort quality is dependent on the enzymes that produce growth, and hence on the degree of modification of the malts. The turbidities decrease and stability increases with increase in salt-soluble nitrogen, malting loss, malt extract, malt saccharogenic activity, and wort nitrogen content and the reverse occurs with increase in steeping time.

## Discussion

The correlation coefficients given in Table I show that wort quality is associated with malt extract, saccharogenic activity (Lintner value), and wort nitrogen content, which are the commonly measured malt properties. However, with one exception, the correlations are not such that any one of these properties can be considered to control any one wort quality. The exception is wort nitrogen content, which accounts for about 70% of the variations in wort viscosity. Partial correlation coefficients were calculated, but they do not contribute to the study except to emphasize the interlocking relations of malt properties to wort quality. For this reason, and also since they may be readily calculated from the simple correlation coefficients, the partial coefficients are not reported.

It is of interest to note that improvements in malt properties are accompanied by improvements in wort quality. These are readily comprehended when considering increases in malt extract and saccharogenic activity, but the increase in wort nitrogen content must be regarded as a qualitative improvement rather than as a strictly quantitative improvement. Low wort nitrogen in a variety is generally accompanied by deficiencies in enzymatic activity. Therefore, among varieties, as enzymatic activities increase, malt extract, saccharogenic activity, and wort nitrogen also increase. These increases cause an improvement in wort quality.

The relations of wort quality to malt extract and Lintner value are particularly interesting in view of the fact that both these properties can be predicted from barley properties. Starch and barley extract are closely related to malt extract, while saccharogenic activity (Lintner value) of the barley after extraction with papain is closely related to Lintner value of the malt (cf. Table II and also 11, 12). However, neither starch, extract, nor Lintner value of barley shows a significant association with any wort quality. That is, there are factors contributing to wort quality that are not measured by barley extractives or saccharogenic activity. But these factors are measured to some extent by malt extract and enzymatic activity. An examination of the relations between barley and malt properties throws some light on the reasons for this.

The determination of starch and extract content of barley provides information on the available material that can be converted into malt extract and there is a close relation between these barley properties and malt extract. However, the differences between varieties in potential extractives do not account for all of the differences between varieties in malt extract. This failure is caused by the differences between varieties in enzymatic activity, and as noted previously, various properties have been examined to determine whether they can be applied to the prediction of malt extract as indices of enzymatic activity (13). This failure of barley properties to reflect enzymatic properties is further seen in the relations between the various saccharogenic activities. The saccharogenic activity (Lintner value) of barley after activation by papain is attributed solely to the action of $\beta$-amylase. While this

activity is closely related to the saccharogenic activity (Lintner value) of the ensuing malt, it may be expected to be more closely related to $\beta$-saccharogenic activity than to total saccharogenic activity (cf. 7). Similarly, while barley Lintner value is related to malt $\alpha$-amylase activity the relation is not as close as that between the Lintner value and the $\alpha$-amylase activity of malt (3). That is, only part of the saccharogenic activity of malt can be predicted from the saccharogenic activity of the barley, since the latter does not provide an adequate estimate for malt $\alpha$-amylase activity.

The differences in the relations of the barley properties and similar malt properties to wort qualities may be explained, therefore, on the basis of enzymes present in the malt, but not in the barley. These enzymes contribute to both the amount of extract and the quality of the wort. Among these it is reasonable to assign a significant role to $\alpha$-amylase. Unfortunately, neither $\alpha$-amylase nor autolytic saccharogenic determinations were made on the malts used in this study.

The complexity of the factors influencing wort quality is evident from the low values of the various correlation coefficients that are statistically significant (Table I) and, as noted previously, no individual barley or malt property can be considered to control any one wort quality, though wort nitrogen content appears to play a major role in determining wort viscosity. Scatter diagrams illustrating the relation between the degree of attenuation and malt extract, malt Lintner value, and wort nitrogen content were prepared. They were unusual in that the degree of scatter about the regression line was not uniform, and the correlation surfaces appeared to be made up of several swarms. The relation between malt extract and degree of attenuation ($r = .572^{**}$) is illustrated in Fig. 1 A. This scatter diagram is the most interesting of the three prepared, and is the only one reproduced in this

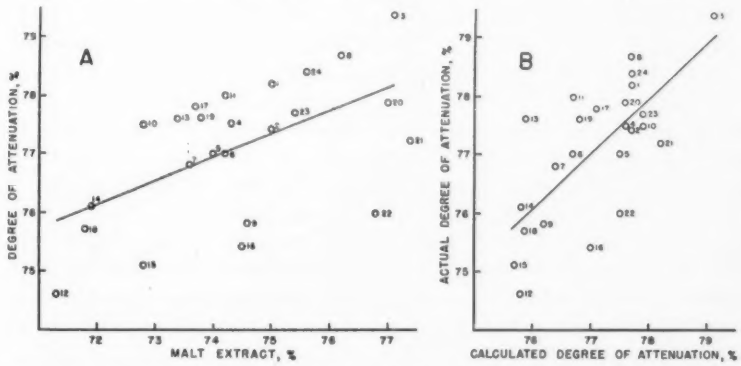


FIG. 1. *Scatter diagrams for varietal means showing the relations between degree of attenuation and malt properties. In Fig. 1 B the relation is that between actual degree of attenuation and values computed from the equation: Attenuation = 49.4 + .33 malt extract + .03 Lintner value. The key to varieties is given in Appendix Table I.*

***Denotes that the 1% level of significance has been attained.*

paper. The 24 varieties appear to be divided into three distinct groups, among which the slopes of the regression lines are similar. The grouping of the varieties is not in accord with any one characteristic; however, inclusion of malt saccharogenic activity (Lintner value) in a multiple correlation modifies the relation appreciably. The multiple correlation coefficient of malt extract and Lintner value with degree of attenuation is $R = .710^{**}$. The calculated values for degree of attenuation, obtained from the equation:

$$\text{Degree of attenuation} = 49.4 + .33 \text{ malt extract} + .03 \text{ malt Lintner value},$$

are plotted against actual values in Fig. 1 B. The scatter is considerably reduced from that in Fig. 1 A. The varieties responsible for the largest residual deviations from the regression line are: Plush, Nobarb, and Brandon 216, for which the actual degree of attenuation is higher than the estimated value; and Ottawa E. 25, Sask. 264, Hannchen, and Victory, for which the actual degree of attenuation is lower than the estimated value. None of the available data will satisfactorily account for the greater deviations of these particular varieties from the regression line. It should be noted, however, that it is an enzymatic property that was applied to the data represented in Fig. 1 A to obtain a better estimate of degree of wort attenuation from malt properties.

The associations of wort qualities with malt extract and wort nitrogen content are similar in character, and they also provide additional evidence on the importance of enzymatic development in determining wort quality. Both malt extract and wort nitrogen content are expressions of two main factors; amount of substrates available and enzymatic activity. The varieties differ more in malt extract and wort nitrogen content than in barley starch, extract, and nitrogen content (see Appendix Tables I and II) and these greater differences are the results of differences in enzymatic activity between the varieties. If the malt extracts of the varieties differed only in quantity, then extent of fermentation and relative viscosity would not change with increase in extract. Similarly, if wort nitrogen increased only in quantity, there would be a decrease in fermentability, due to replacement of carbohydrate with nitrogenous material, and viscosity would increase. But increase in extract and wort nitrogen are accompanied by increase in fermentability and decrease in viscosity. Hence, the varieties differ not only in amount of extract, but also in composition of wort: and between varieties, as extract increases, the molecular size of its components decreases. The increased quantity of extract and wort nitrogen may be caused either by an increase in the available substrates or by an increase in enzymatic activity, but the decrease in molecular size can be caused only by an increase in extent of hydrolysis caused by increased enzymatic activity.

The complexity of the factors controlling wort quality has been pointed out in previous paragraphs, but it is of interest to determine the combined effects of the three major malt properties on wort quality. The multiple correlation coefficient of malt extract and saccharogenic activity (Lintner

## APPENDIX TABLE I

VARIETAL MEANS FOR BARLEY PROPERTIES

| Class | Variety | Total nitrogen, % | Salt-sol. nitrogen, % | Starch, % | Extract, % | 1000-kernel wt., gm. | Sacch., act., °L. | Plump barley, % |
|---|---|---|---|---|---|---|---|---|
| Rough-awned 6-rowed malting | 1. O.A.C. 21 | 2.08 | .54 | 54.1 | 75.9 | 31.8 | 186 | 76.9 |
| | 2. Mensury | 2.06 | .53 | 54.0 | 75.8 | 31.9 | 194 | 78.4 |
| | 3. Olli | 2.00 | .54 | 54.6 | 77.2 | 29.6 | 216 | 68.0 |
| Rough-awned 6-rowed non-malting | 4. Peatland | 2.34 | .56 | 53.6 | 75.8 | 29.2 | 221 | 72.7 |
| | 5. Pontiac | 2.04 | .50 | 53.5 | 74.9 | 31.8 | 193 | 80.6 |
| | 6. Trebi | 1.88 | .46 | 56.2 | 76.8 | 40.0 | 193 | 87.7 |
| Smooth-awned 6-rowed | 7. Alberta 8 | 2.03 | .46 | 54.7 | 74.8 | 33.6 | 153 | 82.6 |
| | 8. Brandon 216 | 1.90 | .47 | 55.6 | 76.7 | 24.4 | 142 | 45.3 |
| | 9. Byng | 1.90 | .43 | 55.6 | 77.6 | 33.9 | 179 | 87.7 |
| | 10. Newal | 2.17 | .50 | 52.6 | 75.5 | 34.2 | 276 | 95.2 |
| | 11. Nobarb | 1.91 | .44 | 55.6 | 76.9 | 32.6 | 158 | 79.3 |
| | 12. Ottawa E. 25 | 2.20 | .44 | 52.3 | 74.6 | 37.6 | 195 | 94.0 |
| | 13. Plush | 1.97 | .41 | 54.8 | 75.9 | 33.8 | 142 | 81.4 |
| | 14. Prospect | 2.03 | .45 | 52.4 | 74.5 | 35.0 | 170 | 92.0 |
| | 15. Regal | 2.07 | .48 | 53.3 | 74.8 | 31.2 | 133 | 75.2 |
| | 16. Sask. 264 | 2.07 | .49 | 53.6 | 75.6 | 32.2 | 159 | 60.5 |
| | 17. Velvet | 2.10 | .47 | 53.6 | 74.9 | 30.6 | 177 | 82.0 |
| | 18. Wisconsin 38 | 2.05 | .44 | 53.2 | 74.6 | 32.8 | 167 | 90.5 |
| | 19. York | 2.08 | .48 | 54.5 | 76.3 | 31.4 | 209 | 78.8 |
| Rough-awned 2-rowed | 20. Charlottetown 80 | 2.05 | .52 | 56.9 | 78.6 | 33.8 | 169 | 82.7 |
| | 21. Hannchen | 1.99 | .50 | 57.3 | 79.3 | 32.6 | 174 | 69.4 |
| | 22. Victory | 1.96 | .49 | 57.1 | 79.1 | 34.4 | 152 | 79.0 |
| Smooth-awned 2-rowed | 23. Rex | 2.17 | .49 | 54.4 | 76.5 | 36.7 | 179 | 82.2 |
| | 24. Sanalta | 2.13 | .47 | 55.6 | 77.4 | 39.2 | 180 | 95.6 |
| Mean over all varieties | | 2.05 | .48 | 54.6 | 76.3 | 33.1 | 180 | 79.9 |
| Necessary difference, 5% level | | 0.12 | .03 | 1.2 | 1.0 | 2.1 | 21 | 10.9 |

value) with degree of attenuation is $R = .710^{**}$, while that of malt extract and wort nitrogen with viscosity is $R = .873^{**}$. The addition of wort nitrogen to the former and of Lintner value to the latter multiple relation resulted in no significant increases in the coefficients. The best estimates of initial and final turbidity, and of stability, from malt properties are obtained from the simple regression coefficients involving these properties and wort nitrogen content. The additions of malt extract and Lintner value to the estimation of these did not improve the estimates significantly. The associations of wort nitrogen content with turbidity and stability are loose, though significant. Since wort viscosity decreases with increase in wort nitrogen content, low wort nitrogen is indicative of incomplete degradation of protein material with the resultant presence of protein particles of colloidal size. Therefore the loose associations between wort nitrogen content and turbidity are in accord with the belief that protein material of high molecular weight is partially responsible for turbidity (9, and papers cited therein).

***Denotes that the 1% level of significance has been attained.*

## APPENDIX TABLE II

VARIETAL MEANS FOR MALTING AND MALT PROPERTIES

| Class | Variety | Malting property | | | Malt property | | |
|---|---|---|---|---|---|---|---|
| | | Steeping time, hr. | Malting loss, % | Sprouts, % | Extract, % | Wort nitrogen, % | Sacch. activity, °L. |
| Rough-awned 6-rowed malting | 1. O.A.C. 21 | 52 | 8.2 | 3.8 | 75.0 | 1.06 | 116 |
| | 2. Mensury | 55 | 7.7 | 3.6 | 75.0 | 1.07 | 118 |
| | 3. Olli | 38 | 8.4 | 4.0 | 77.1 | 1.11 | 143 |
| Rough-awned 6-rowed non-malting | 4. Peatland | 59 | 8.1 | 3.8 | 74.3 | 1.10 | 124 |
| | 5. Pontiac | 56 | 7.7 | 3.4 | 74.0 | 1.02 | 124 |
| | 6. Trebi | 64 | 6.6 | 2.6 | 74.2 | 0.80 | 92 |
| Smooth-awned 6-rowed | 7. Alberta 8 | 60 | 7.5 | 3.4 | 73.6 | 0.96 | 89 |
| | 8. Brandon 216 | 43 | 8.2 | 4.2 | 76.2 | 1.04 | 106 |
| | 9. Byng | 55 | 6.3 | 2.6 | 74.6 | 0.76 | 74 |
| | 10. Newal | 53 | 6.6 | 2.6 | 72.8 | 1.00 | 149 |
| | 11. Nobarb | 59 | 7.2 | 3.2 | 74.2 | 0.83 | 93 |
| | 12. Ottawa E. 25 | 56 | 6.3 | 2.8 | 71.3 | 0.87 | 96 |
| | 13. Plush | 58 | 6.4 | 2.7 | 73.4 | 0.81 | 76 |
| | 14. Prospect | 56 | 6.9 | 3.0 | 71.9 | 0.84 | 91 |
| | 15. Regal | 55 | 7.2 | 3.0 | 72.8 | 0.95 | 77 |
| | 16. Sask. 264 | 50 | 8.6 | 4.0 | 74.5 | 1.08 | 100 |
| | 17. Velvet | 56 | 7.4 | 3.4 | 73.7 | 1.04 | 114 |
| | 18. Wisconsin 38 | 62 | 7.4 | 3.1 | 71.8 | 0.86 | 93 |
| | 19. York | 66 | 6.5 | 2.4 | 73.8 | 0.90 | 101 |
| Rough-awned 2-rowed | 20. Charlottetown 80 | 56 | 8.5 | 4.0 | 77.0 | 0.96 | 94 |
| | 21. Hannchen | 52 | 8.3 | 3.6 | 77.4 | 1.00 | 111 |
| | 22. Victory | 56 | 7.9 | 3.6 | 76.8 | 0.86 | 93 |
| Smooth-awned 2-rowed | 23. Rex | 58 | 8.0 | 3.6 | 75.4 | 1.12 | 119 |
| | 24. Sanalta | 48 | 7.7 | 3.3 | 75.6 | 1.04 | 112 |
| Mean over all varieties | | 55 | 7.5 | 3.3 | 74.4 | 0.96 | 104 |
| Necessary difference, 5% level | | 6 | 0.6 | 0.4 | 1.1 | 0.06 | 12 |

From the data obtained in this investigation, it is obvious that measurements of malt extract, Lintner value, and wort nitrogen content of malt do not provide adequate means for assessing wort quality, though they do provide indications. It must be concluded that varieties that are promising with respect to commonly measured malt properties should be tested further by means of wort quality determinations. However, as has been indicated (9), varieties that are deficient in the commonly measured barley or malt properties are considered as unsuitable for malting, and their wort qualities need not be examined. However, the study of varieties that are known to have undesirable qualities has brought to light relations that would otherwise be difficult to detect, and these are capable of practical application.

It is an axiom in brewing that beer is never better than the malt from which it was made. In spite of the differences in opinion, mentioned in a previous paper (9), as to whether determinations made on laboratory worts bear any relation to qualities of commercial hopped and boiled worts and beers, it

seems worthwhile to consider the data presented in this paper in relation to commercial brewing. Actually, it is impossible to prove conclusively that laboratory worts indicate brewing value unless brewing trials are conducted. These are not possible at present in Canada. However, in view of the relations between the wort qualities and barley and malt properties described herein, certain observations are pertinent.

Among the 24 varieties studied, the wort qualities—degree of attenuation, viscosity, and turbidity—are related to malt extract, malt saccharifying activity, and wort nitrogen, and to barley salt-soluble nitrogen, steeping time, and malting loss. The relations are not particularly close, but they are statistically significant. Further, the wort qualities are not related to barley extract, starch, or $\beta$-amylase activity after activation by papain. Therefore, the wort qualities appear to be related to enzymes other than $\beta$-amylase that are developed during malting and that change the barley materials into malt extract during mashing. These wort qualities also reflect the extent to which starch and protein are degraded in the wort and are indicative of molecular size and type. If the molecular size and type of the wort solids are dependent on the enzymatic properties of the malt, a similar condition should obtain in commercial worts, and it seems likely that quantitative and qualitative differences, particularly in fermentability and viscosity, would persist in some degree through boiling, hopping, and fermentation, into the beer.

The interpretation of haze in laboratory wort is complicated by the fact that, in the course of boiling, hopping, and fermentation of worts in commercial brewing there are successive precipitations of colloidal material. However, Canadian maltsters and brewers regard with suspicion any malt that produces a turbid laboratory wort. Our results indicate that haze in laboratory wort, when varieties are being compared, is associated with low extract, low saccharifying activity, low wort nitrogen, low degree of attenuation, and high viscosity. Thus, haze appears to be the result of inadequate malt modification. While turbidity in commercial wort may not be caused by identically the same material that causes turbidity in laboratory wort, it is probable that they stem from a common source. At least, haze in a laboratory wort is indicative that there is a lack of satisfactory balance of properties in the malt.

## Acknowledgments

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# SEPARATION OF STARCH AND GLUTEN

## I. DEVELOPMENT OF MECHANICAL EQUIPMENT AND WASHING METHODS FOR SEPARATION FROM WHEAT FLOUR[1]

By G. A. Adams[2], G. A. Ledingham[3], and N. H. Grace[2]


## Abstract

Starch and gluten were separated from wheat flour in a washing vessel, hemicylindrical in shape, oscillated 48 times per minute through 45°, accommodating 15 kgm. of water plus the dough prepared from 3 kgm. of patent hard spring flour. Washing action was provided by the combined movements of the vessel and an adjustable free-swinging rubber-covered roller. The starch suspension was continuously pumped into a settling tray, from which the overflow was recycled to the washing machine. Under these conditions 45 min. of washing with 40 kgm. of water effected a recovery of about 95% of the starch and gluten present in the dough. About 70% of the starch was extracted in the first 10 min. washing period.

Washing was more efficient at 37° C. than at lower temperatures, and dough mixing times of nine minutes gave better results than three minutes. The washing equipment provided results of a precision that permitted demonstration of statistically significant differences between starch yields differing by as little as 3%.


## Introduction

Accumulation of wheat surpluses prior to the present war directed attention to the wider utilization of wheat products in industry. Although the production of starch from wheat is undoubtedly one of the oldest branches of the starch industry, technical and scientific advances have been concentrated on starch production from cheaper sources. Since gluten, the major protein fraction, makes up approximately 15% of wheat flour, successful commercial development may largely depend on the utilization of this fraction. Gluten has been used mainly by food industries, for which purpose the presence of considerable starch has not been a particularly serious disadvantage. However, in view of the possible demand for this protein as a basic raw material in industry, its relative freedom from starch may become more important. Hence improvements in methods of separation leading to rapid and efficient production of both relatively pure gluten and starch are desirable.

Either ground whole wheat or commercial wheat flours could be used as raw material from which to obtain starch and gluten: however, this investigation was confined to straight run commercial flours, as it was believed simpler to work with a product from which the bran, shorts, and germ had been removed.

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## Development of Equipment and Methods of Washing

Several processes for the commercial preparation of wheat starch are known. The earliest method, known as the Halle or fermentation process (4), depends on the partial fermentation of the gluten fraction of wheat with the consequent liberation of the starch. The Alsatian process (7) employs a kneading action on ground wheat in a trough of water. It has the advantage of yielding a good grade of gluten, but the separation is time-consuming and inefficient. The Martin process (5), which is in current use, involves the mechanical washing of a stiff dough prepared from flour. A good grade of both starch and gluten can be prepared by this method. The Fesca process (6) also uses flour as the raw material and separates the starch from the gluten by means of a continuous centrifuge. This method is economical but yields a gluten high in starch. The recovered starch has a relatively high nitrogen content and requires considerable purification before use for some purposes. None of these methods has been particularly satisfactory; the fermentation process destroys most of the gluten fraction, and existing mechanical methods of separation are rather inefficient.

In laboratory investigations, both the dough-mixing and washing processes have usually been designed to do as little work as possible on the gluten in order to maintain its baking qualities. In general, these methods yield a gluten with a high starch content. Gluten intended for such industrial uses as the making of sodium glutamate, plastics, paints, and fibres should be free from starch and is not likely to be adversely affected by the more drastic washing treatments necessary.

Development of efficient mechanical washing equipment and procedures involved numerous preliminary studies of possible types of washing actions. Among these various motions were those afforded by a rotary churn, a gyrator type laundry washing-machine, a rotary gear pump, and finally, mechanical dough-kneading equipment. None of these washing methods except that based on the mechanical dough-kneading principle was readily adaptable to the continuous removal of relatively gluten-free starch suspension.

The investigation of the kneading principle of washing led to the construction of several different machines. In the first of these the charge of dough was placed on the curved screen base of a hemicylindrical vessel and worked back and forth under a spray of water by a cam driven roller. There was a tendency for small particles of gluten to block the screen or to be washed through with the starch suspension. Modified machines with sheet metal bottom and screen mesh on the sides eliminated part of this difficulty, but washing was slow and too much gluten still escaped with the starch. As a further modification, a rocking motion was imparted to a metal washing vessel (volume 50 litres) by oscillating it through 45° by an eccentric drive. This method was also slow and inefficient. However, when a freely swinging wooden roller was suspended in the vessel, the resulting kneading action accelerated washing. Tests were then made on many different types of roller. Manual adjustment of roller tension by means of screws proved to be too variable.

Finally a mechanical control was developed for gradually lowering the roller and thereby applying increased kneading action during the washing operation.

The essential features of this mechanism are shown in Figs. 1 and 2. The roller is approximately 4 in. in diameter, constructed of wood covered with corrugated rubber, and moving freely on suitable bearings. Fig. 2 shows in cross section the nature of the suspension of the roller. The diagram shows the roller in its lowest position, where it exerts the maximum working

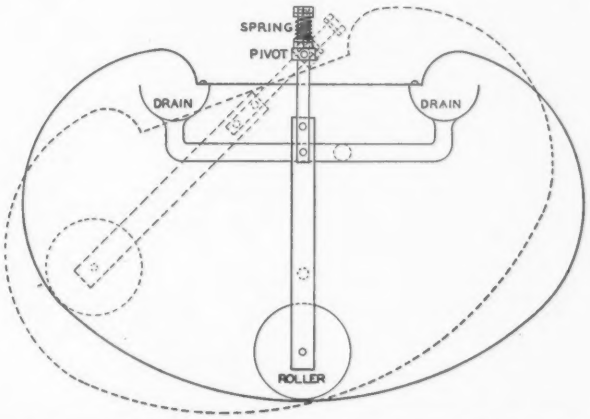


FIG. 1. *Cross section of hemicylindrical washing vessel, free swinging roller, and drains into which starch suspension is splashed.*

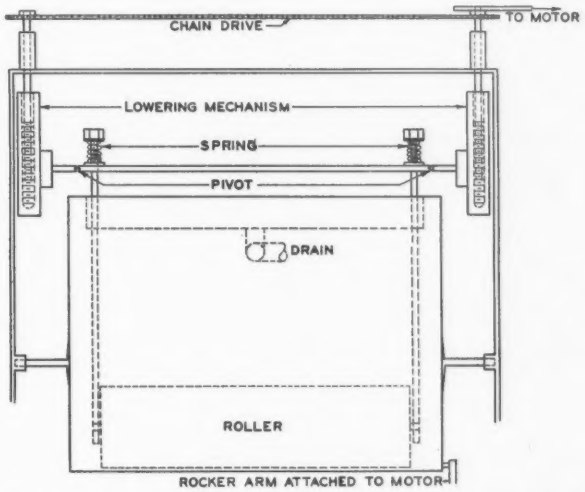


FIG. 2. *Cross section illustrating mechanism for lowering roller, which is shown in its lowest position.*

pressure. Adjustment of the gear ratio of the dropping mechanism permits any desired rate of lowering, to give increasing pressure on the gluten.

The charge of dough is placed in the drum in approximately 15 kgm. of water with the roller in the uppermost position, i.e., exerting slight pressure. The roller is gradually lowered, reaching the position of maximum working pressure after 20 min. The rocking motion, 48 complete excursions to the minute, splashes starch suspension up to the two metal drains on the upper inside edge of the vessel (Fig. 1), the drains being emptied by a pump. The volume of suspension was maintained at approximately 15 kgm. in the washing vessel by the addition of either fresh water or liquor from which most of the starch had been removed. Fig. 3 shows the equipment as set up for operation.

Starch has been successfully separated from water suspensions by settling in a tank, by the use of a continuous centrifuge, and by passing over trays. Work with experimental settling tanks showed that considerable time was required for complete settling and a relatively large volume of water was necessary. A continuous centrifugal method of separation required less water than the tank and tray methods. However, owing to the accumulation of soluble material in the wash water, recirculation of a small volume of water (15 to 20 kgm.) brought about an undesirable dispersion of the gluten. The tray method appeared to be the most promising.

The trays used were 16 ft. long, 8 in. wide, and 4 in. deep. The starch suspension was passed over three trays, arranged with a fall of from $\frac{1}{4}$ to $\frac{1}{2}$ in. per 16 ft. It was found that the bulk of the starch came out in the first 10 ft. of tray and use of more than one tray was then discontinued. The tray system of separation has been modified to permit both tabling and settling: to accomplish the latter the tray was maintained level and dammed at the end. Starch suspension was poured in at one end and flowed out at the other while a depth of liquid of about 2 in. was maintained in the tray. The overflow was circulated back to the washing equipment. In most of these washing experiments the initial amount of water was 40 kgm., 15 of which was placed in the washing machine and the balance in the tray. When the washing operation was terminated all the wash water, including 10 kgm. used for a final rinse of the gluten and machine, was pumped into the tray and held there for a period of about three hours to permit settling of the small amount of starch still in suspension. Maintenance of a constant washing temperature was achieved by covering the trays and recirculating the liquor through coils immersed in a constant temperature bath.

## Methods

### *Intermittent Washing*

These washing experiments were made before final modification of the washing equipment. Provision was not made for the gradual lowering of the roller: it was set in the 'down' position (Fig. 2) and its pressure regulated by spring action.

Dough was prepared in a laboratory mixer from 3 kgm. of flour and 1.9 kgm. of water and placed in the washer with 16 kgm. of water. The temperature

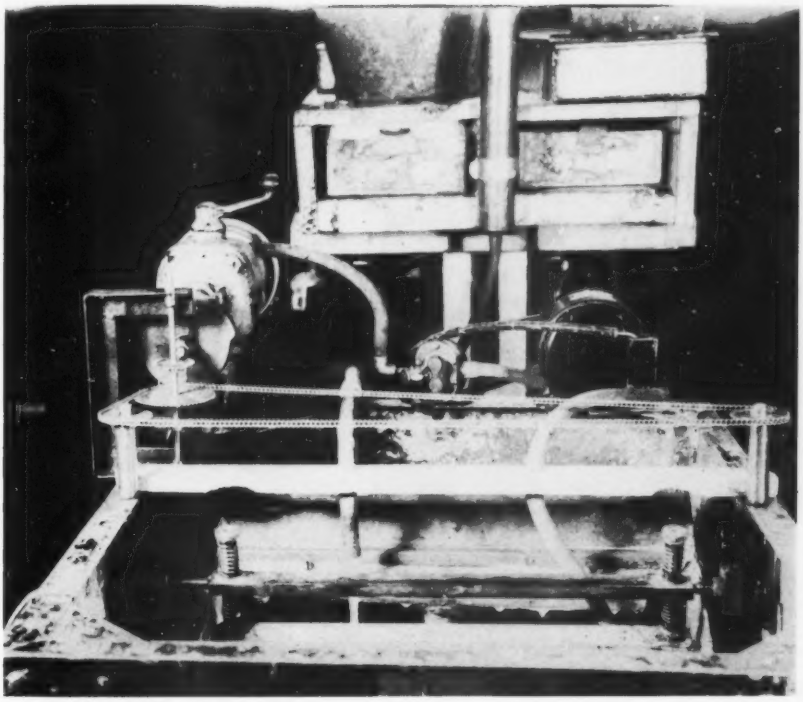

FIG. 3. *View of washing equipment in operation showing lowering mechanism, pump, starch trays, and upper portion of hemicylindrical washing vessel.*

of the mixing and washing water was 25° C. After a 10 min. washing period the starch suspension was drawn off through a 30 mesh copper screen, which retained small pieces of gluten. Sixteen kilograms of fresh water was then added and the procedure repeated four times. The four combined starch suspensions were pumped over three 16 ft. tray sections, or alternatively allowed to collect in one section equipped with a closed end containing a series of stoppered holes ½ in. apart vertically. The removal of the stoppers at intervals of 40 min. resulted in a supernatant liquor that contained very little starch. In all, a series of 19 washing experiments was made by this intermittent method.

*Continuous Washing*

The four experiments about to be described in detail all involved a washing period of 45 min. and recirculation of 40 kgm. of water at a rate of 2 kgm. per minute with a two minute final rinse of the gluten in 10 kgm. of fresh water, in the equipment shown in Figs. 1, 2, and 3.

The factors investigated in the four series of experiments are listed in Table I. Unless otherwise specified, the water used for mixing the dough and for the conditioning was maintained at the temperature of the wash water.

TABLE I

FACTORS INVESTIGATED IN WASHING EXPERIMENTS†

| Experiment No. | Dough mixing time, min. | Dough curing time, min. | Washing temp., °C. | No. of separations |
|---|---|---|---|---|
| 1 | 3, 6 | 0, 15, 30 | 27, 37 | 24 |
| 2 | 3, 6 | 0, 15, 30 | 37 | 12 |
| 3 | 3, 6, 9 | 0, 30 | 27, 37 | 23†† |
| 4 | 3, 6, 9 | 0, 30 | 27 | 12 |

† *In all four experiments 1800 and 2000 ml. of water were used with 3 kgm. of flour, providing two dough consistencies.*

†† *One missing separation.*

The effect of these variables on washing was ascertained by determination of the amount of recovered gluten and starch. A record was made of the wet weight of gluten following a draining period of 15 min.; the gluten was then put through a rotary drum drier, ground, weighed, and analysed for starch and nitrogen content. The starch collected in the trays was dried in a tunnel drier for approximately 24 hr. at 40° C.; ground, weighed and analysed for starch and nitrogen content; and some ash determinations were made. Soluble nitrogen and residual starch were determined in some of the tailing waters.

In Experiment 1, the roller was operated for five minutes without tension or in the 'up' position, and then lowered to the half-way position. The

washing continued for 10 min. under the increased tension. Then the roller was dropped to the 'down' position in which full pressure was applied and maintained for the balance of the washing period, namely, 30 min. During Experiments 2 to 4 the roller tension was mechanically controlled as has been described.

*Preliminary Separations at 17° C.*

A number of isolated separations were made at this temperature. Some of the variables considered were higher temperatures in mixing dough (37° C.), conditioning at 37° C., and substantially increased mixing and washing times.

*Chemical Analyses*

Starch in flour and gluten was determined by the polarimetric method developed in these laboratories (2,3). Moisture was determined in the flour and starch by drying in a vacuum oven for 16 hr. at 105° C.; for gluten the drying time was extended to 24 hr. The Kjeldahl procedure was used for nitrogen determinations. Ash and fat were estimated in flour, starch, and gluten by standard methods (1). All analytical results are expressed on a moisture-free basis. The pH of the tap water used in all the experiments ranged from 7.0 to 8.5.

Supplies of flour sufficient to carry out the entire set of experiments were mixed, sampled, and analysed at the beginning of the investigations. The results are shown in Table II.

TABLE II

ANALYSIS OF THE FLOURS USED IN WASHING EXPERIMENTS, %

| Method of washing | Moisture | Starch | Nitrogen | Protein (N × 5.7) | Ash | Fat |
|---|---|---|---|---|---|---|
| Intermittent | 11.80 | 68.23 | 2.25 | 12.72 | 0.40 | 0.75 |
| Continuous | 12.20 | 68.20 | 2.32 | 13.22 | 0.42 | 0.99 |

## Results

*Intermittent Washing*

The results are shown in Table III. It is apparent from the average results in Table III*A* that 60% of the available starch settles out on the first tray. A lower quality of starch is obtained on the other two trays. Since only 70% of the starch was recovered from the continuous flow over three trays, it was found advantageous to use one tray for settling with periodic drainage of the supernatant solution. Average results are shown in Table III*B*. Approximately 87% of the starch in the flour is recovered by this method. 'Satisfactory' washing experiments yielded gluten with about 10% starch content: 'unsatisfactory' separations yielded gluten with a starch content ranging from 25 to 30%. Average gluten recovery was about 95% on a

TABLE III

ANALYTICAL RESULTS FOR STARCH AND GLUTEN
(From 3000 gm. flour (11.8% moisture))

| | Yield, gm. | Nitrogen content, % | Starch content, % | Yield from flour, % |
|---|---|---|---|---|
| *A. Starch collected on three trays* | | | | |
| Starch | | | | |
| Tray 1 | 1348 | 0.097 | 95.90 | 60.60 |
| Tray 2 | 137 | 0.327 | 85.66 | 5.8 |
| Tray 3 | 83 | 0.368 | 84.46 | 3.5 |
| Gluten | 435 | 12.90 | 12.2 | 95.80 |
| *B. Starch collected on one tray* | | | | |
| Starch | 1778 | 0.133 | 91.89 | 87.72 |
| Gluten† | 412 | 13.40 | 10.1 | 94.6 |
| Gluten†† | 540 | 10.74 | 28.8 | 96.9 |

† *Gluten from nine satisfactory washing experiments.*
†† *Gluten from two unsatisfactory washing experiments.*

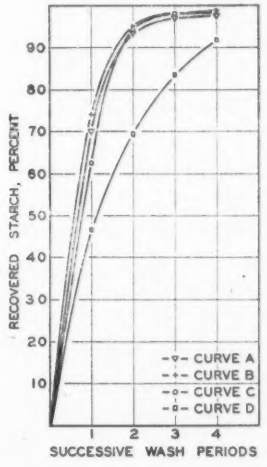


FIG. 4. *Percentage of starch extracted from flour dough by four successive 10 min. washing periods.*

starch-free basis, and it was not appreciably influenced by the amount of residual starch.

The amount of starch extracted by each of the four successive 10 min. washing periods is shown in Fig. 4. Satisfactory separations (Curves *A*, *B*, and *C*) show that about 70% of the recovered starch is extracted by the first

10 min. washing period. Furthermore the last 10 min. washing period removed only 0.5% of the starch. Curve *C* represents the effect of slowing the rocking motion of the machine from 48 to 40 oscillations per minute: it is apparent that extraction was reduced during the initial 10 min. period but increased during the second period: the total extraction was not impaired. In Curve D the results of a typically poor separation are shown: the starch recovery was low.

Fig. 4 shows the cumulative extraction of starch by four washing periods. Fig. 5 presents the percentage starch extraction in each period based on the amount present at the beginning of that period. Direct comparison of the rate of starch removal for each working period is thereby permitted. The removal of starch follows essentially the same pattern in Curves *A* to *C*.

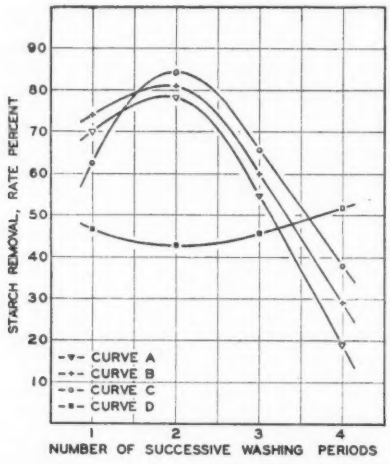


FIG. 5. *Starch removed during each 10 min. washing period expressed as a percentage of that present at the start of the period.*

The rate of removal for these three separations attained a maximum at the second period and thereafter decreased approximately linearly. The maximum rate is reached at the point where about 90% of the starch has been removed: up to this point, the dough was soft, with little coherence, and tending to break into fragments favouring rapid removal of the starch. On the other hand, when more than 90% of the starch has been removed, the starch to gluten ratio is reversed and the gluten is coalesced into a ball. Consequently the rate of removal of the starch embedded in a preponderance of gluten becomes a direct function of time and work. Comparison of Curve *C* with *A* and *B* illustrates the effect of reducing the oscillation rate of the washer from 48 to 40 per minute. There is no marked change in the extraction pattern. The increased rate of starch removal during the second period (Curve *C*) may be related to the reduced work and extraction that occurred in the first

period. Not only was there more starch present to be removed but the dough had been worked into a condition that favoured easy and rapid starch removal. Less rapid decline in the linear region of the curve is directly related to the reduced work applied.

An entirely different mechanism of starch removal was shown by the unsatisfactory separation (Curve *D*). Such separations have been characterized by a partial breakdown of the dough into relatively coarse stringy aggregates. These glutenous masses offer reduced surface for washing action as compared with the small nodular pieces of dough obtained in satisfactory separations. Curve *D* shows a decline in removal rate between the first and second periods and thereafter a slight increase, corresponding to the gradual coalescing of the coarse dough particles to provide a coherent mass upon which direct kneading action can be imposed.

While the intermittent method of washing yielded a good quality of gluten it had certain disadvantages, notably the difficulty of screening the starch suspension at the end of each washing period. This difficulty was obviated by the adoption of a continuous washing procedure.

*Continuous Washing* (The factors investigated are listed in Table I.)

*Experiment 1*

Washing tended to be erratic in Experiment 1 and consequently complete chemical analyses of all starch and gluten samples were not warranted, though they were made for some of the more satisfactory separations. These results showed a residual starch in gluten of approximately 10% and about 0.11% nitrogen in the starch. A significant feature of the results was the superiority of separations made at 37° C. The results of the 27° C. washing showed a high degree of variability. Statistical treatment of the data was not made.

In Table IV, data are given for the results of statistical treatment of the analytical results of Experiments 2 to 4. The means of some of these results are given in Table V.

*Experiment 2*

The consistency of the dough and conditioning affected the wet weight of gluten. The 2000 ml. consistency increased the wet gluten weight by about 4% and about the same increase followed each of the 15 and 30 min. curing times, which did not differ significantly between themselves. However, there were no such effects on the dry weight of gluten, indicating that merely hydration or imbibition phenomena were involved. The average value for nitrogen in starch was 0.138%.

*Experiment 3*

Washing temperature affected the wet weight of the gluten, the percentage of nitrogen found in the starch, and the total yield of starch in Experiment 3. The only other statistically significant observation was the effect of curing time on the solubility of nitrogen in the tailing water. The average weight

## TABLE IV

ANALYSES OF VARIANCE OF CONSTITUENTS OF DOUGH SUBJECTED TO VARIOUS PROCESSING CONDITIONS

| Source of variance | Degrees of freedom | Mean square | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Wet weight of gluten | Moisture-free weight of gluten | Nitrogen in gluten | Starch in gluten | Nitrogen in starch $\times 10^3$ | Yield of starch | Soluble nitrogen in tailings |
| Experiment 2 | | | | | | | | |
| Mixing times | 1 | 140 | 61 | 0.1452 | 11.21 | 0.385 | | |
| Consistencies | 1 | 8164** | 520 | 0.2409 | 3.00 | 0.005 | | |
| Curing times | 2 | 2553* | 77 | 0.0083 | 9.43 | 0.280 | | |
| Mixing time × consistency | 1 | 271 | 91 | 0.0050 | 10.09 | 0.433 | | |
| Mixing time × curing | 2 | 525 | 712 | 0.0367 | 4.08 | 0.186 | | |
| Consistency × curing | 2 | 349 | 72 | 0.0420 | 8.20 | 0.202 | | |
| Mixing time × consistency × curing | 2 | 31 | 27 | 0.2259 | 0.52 | 0.387 | | |
| Error | 7 | 297 | 245 | 0.0871 | 5.10 | 1.208 | | |
| Experiment 3 | | | | | | | | |
| Temperature | 1 | 24.962** | 216 | 0.443 | 20.15 | 20.417** | 2817* | 1.591 |
| Mixing time | 2 | 1616 | 549 | 0.499 | 13.84 | 0.487 | 1164 | 2.417 |
| Consistency | 1 | 9441 | 104 | 0.001 | 2.47 | 4.320 | 193 | 1.984 |
| Curing time | 1 | 2204 | 8 | 0.1204 | 18.90 | 7.921 | 704 | 7.172* |
| Error† | 17 | 2149 | 228 | 0.1841 | 8.03 | 1.941 | 532 | 1.362 |
| Experiment 4 | | | | | | | | |
| Replicates | 1 | 1204 | 4428** | 0.0000 | 6.00 | 10.417 | 19,097** | |
| Mixing times | 2 | 10,940 | 668 | 0.5844 | 45.88* | 2.799 | 2468 | |
| Consistency | 1 | 8140 | 8 | 0.0888 | 0.96 | 8.817 | 210 | |
| Curing time | 1 | 37,604** | 726 | 0.0096 | 5.41 | 50.600** | 155 | |
| Mixing times × consistency | 2 | — | — | — | — | — | 6108* | |
| Error† | 17 | 3321 | 293 | 0.2176 | 12.32 | 3.329 | 1247 | |

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
† *One missing value calculated.*

## TABLE V

AVERAGE YIELDS AND PURITY OF GLUTEN

| Experiment number | Dry weight of gluten from 3000 gm. flour | Nitrogen in gluten, % | Starch in gluten, % |
|---|---|---|---|
| 2 | 425.4 | 13.62 | 14.45 |
| 3 | 418.4 | 13.82 | 12.78 |
| 4 | 429.0 | 13.68 | |

of wet gluten was approximately 4% greater for the 37° C. washing temperature. The average nitrogen content of the starch was 0.19% for 27° C. separations and 0.13% for separations at 37° C. Starch yield was 1909 gm. at the lower temperature and 1930 gm. at the higher, with 19.9 as the necessary difference for the 5% level of significance. Soluble nitrogen in the tailing water from dough without curing was 20.3 mgm. per 199 gm., and 21.4 mgm. when the dough had been cured in water for 30 min. prior to washing.

*Experiment 4*

Statistical treatment of the data includes as duplicates, the separations at 27° C. already treated under Experiment 3. Curing affected the wet weight of the gluten and the nitrogen in the starch. The amount of starch retained in the gluten was influenced by the dough mixing time. Yield data on starch indicated that there was a significant interaction between mixing time and dough consistency.

The 30 min. curing of the dough increased the wet weight of the gluten by approximately 7%, and yielded starch with an average nitrogen content of 0.16%, whereas the corresponding value in the absence of curing was 0.25%.

The average percentage of starch retained by the gluten after mixing the dough for three, six, and nine minutes was respectively, 15.7, 15.9, and 11.6; with 3.70 the necessary difference corresponding to the 5% level of significance.

The nature of the interaction between mixing times and consistencies on the total yield of starch was such that the yield was essentially the same for three and six minute mixing with both consistencies, while the 2000 ml. consistency gave a higher starch yield than the 1800 ml. when used in conjunction with the nine minute dough mixing time. The nine minute mix with the lower consistency yielded an average of 1922 gm. of starch, while with the higher consistency the yield was 1987 gm. The average for the whole experiment was 1937 gm. of starch from 3 kgm. of flour.

*Separations at 17° C.*

The average starch content of the gluten from four separations at 17° C. was upwards of 20%. This was reduced to 10% when the dough was prepared with water at 37° C. instead of 17° C. Curing at 37° C. for periods up to one hour also had a beneficial effect. These preliminary results indicated that washing at 17° C. was not practical, unless used in conjunction with higher temperatures during the dough preparation, longer mixing and curing times, and increased washing periods.

While the main purpose of the present work has been development of equipment and methods, some of the results on washing are worthy of comment. Total starch recovery for the better separations was of the order of 95 to 97%, while gluten recovery ranged around 95%. Starch purity varied widely with the particular washing conditions; nitrogen content ranging from 0.1 to 0.2%. However, simple re-washing of the starch and the use of

dilute ammonia or sulphur dioxide treatments have reduced the nitrogen content to a very low level. While the data indicated residual starch and gluten in the better separations to an extent of 10%, gluten recovery is relatively independent of starch content. The washing equipment provided results of a precision that permitted demonstration of statistically significant differences between starch yields differing by as little as 3%.

## Recommended Procedure

The dough washing equipment consisted of an oscillating hemicylindrical vessel (volume approximately 50 litres) equipped with an adjustable roller. It was found to give satisfactory separation of starch and gluten from batches of 3 kgm. of wheat flour.

A stiff dough was prepared in a laboratory dough mixer by kneading the flour with 60 to 65% of its weight of water for nine minutes. The ball of dough may be conditioned for 30 min. (but this is not essential) and kneaded in the washing equipment in approximately 15 kgm. of water. The temperature of the mixing and washing water was maintained at 37° C. A total of 40 kgm. of water was sufficient for the entire process.

Starch suspension was continuously removed to settling trays: fresh water or the effluent from the settling trays was continually supplied to the washing vessel. During the early stages of washing slight roller pressure was applied to the dough: this was gradually increased as the separation progressed. This separation requires 45 min.

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# SEPARATION OF STARCH AND GLUTEN

## II. EFFECTS OF PROCESSING FACTORS ON THE STARCH CONTENT OF WHEAT GLUTEN[1]

By N. H. Grace[2] and K. A. Clendenning[2]


### Abstract

The effects of dough mixing time, dough conditioning, and washing temperature and time upon the mechanical separation of starch and gluten were studied in duplicated factorial experiments in which straight and clear grades of hard red spring wheat flour were employed. Glutens of low starch content were obtained over a considerable range of processing conditions, washing time being the factor of greatest importance. After comparable washing periods of 45, 60, and 90 min., average starch contents were 7.3, 2.4, and 0.8% respectively. The practicability of employing water recirculation in the batch preparation of practically starch-free gluten has also been demonstrated.


### Introduction

Although numerous processes for the separation of starch and gluten from wheat flour have already been described, it has yet to be shown that any of these are capable of yielding practically starch-free gluten. At the present early stage of the development of gluten as an industrial commodity, it is difficult to assess the importance of residual starch content. Starch contents of 10% or even higher should not constitute a serious disadvantage when the gluten is to be used in the food industries; however, the requirements may prove to be more exacting for other uses.

An earlier communication has described a mechanical dough washing device that was developed in these laboratories as a means of separating starch and gluten from wheat flour doughs (1). The approximate range of conditions under which this separation is effected satisfactorily and the yields and reproducibility of the results have also been indicated. The starch content of the gluten fraction, however, exceeded 10% of the oven-dry weight. This earlier work has now been extended to include a study of the conditions under which substantially starch-free gluten may be obtained with the same mechanical dough-washing device.

### Experimental

The flours employed in this study were straight and clear commercial grades prepared from hard red spring wheat (Table I). Uniform doughs were prepared in a Hobart dough mixer from 3000 gm. flour and 1800 gm. water. The dough mixing and conditioning temperatures were the same as in the subsequent dough washing treatment. The doughs were washed in a mechanical device, already described, under conditions that had proved most effective

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[2] *Biochemist, Industrial Utilization Investigations.*

TABLE I

CHEMICAL COMPOSITION OF WHEAT FLOURS, %
(AIR-DRY BASIS)

| Grade | Protein (N × 5.7) | Ash | Starch (polarimetric) |
|---|---|---|---|
| Straight | 13.7 | 0.48 | 67.3 |
| Clear | 15.0 | 0.70 | 62.9 |

in earlier work (1). Unless otherwise stated, fresh tap water or water recirculated from the settling trays entered the washing vessel to compensate for the starch milk continuously withdrawn. The efficiency of the washing techniques under test was judged by the starch content and moisture-free weight of the gluten fractions obtained. Hereafter, the analytical data are reported on a moisture-free basis: methods of drying the gluten (1) and of measuring its starch content (3) have been described elsewhere.

*Series A*

This factorial series, which was duplicated throughout, was designed to test the effects of dough mixing time, dough conditioning, washing time, washing temperature, and flour quality upon the residual starch content of the gluten (Table III). During the dough washing operation, fresh tap water, having a pH of about 8.0, was added and starch suspension was withdrawn from the washer at a constant rate of 2 kgm./min.

*Series B*

This series was designed to test recirculation and other methods of effecting a reduction in the over-all water requirement. The clear flour was mixed in the usual proportion with water for a standard period of three minutes. The doughs were washed immediately for periods of 45 and 60 min. at 27° C. In 12 separations, metered additions of fresh water were made during the first 15 min. of washing. In the remaining four separations, water was not added nor starch suspension removed during the first 10 min. Some of these experiments involved recirculation of supernatant water from the settling trays after an initial 15 min. washing period, while in others recirculation was discontinued and fresh water was added for the final 15 min. of dough washing. The effects of removing starch from the recirculated wash water were also tested by passing the supernatant water from the settling trays through an efficient bowl centrifuge before returning it to the dough washer.

## Results

*Series A*

In Table II are given the results of the analyses of variance of the weight of gluten, weight of gluten on a starch-free basis, and starch content of gluten. Gluten weights were greatly affected by the flour used, and the percent of

TABLE II

ANALYSES OF VARIANCE OF WEIGHT OF GLUTEN, STARCH-FREE WEIGHT OF GLUTEN, AND STARCH CONTENT OF GLUTEN PRODUCED FROM WHEAT FLOUR DOUGHS†

| Source of variance | Degrees of freedom | Mean square | | |
|---|---|---|---|---|
| | | Weight of gluten | Starch-free wt. of gluten | Starch content of gluten |
| Replicates | 1 | 819.4 | 588 | 1.025 |
| Flours | 1 | 47,360.6*** | 47,198*** | 0.544 |
| Conditioning time | 1 | 656.6 | 371 | 2.441 |
| Mixing time | 1 | 19.1 | 163 | 0.406 |
| Washing time | 1 | 2036.3 | 380 | 42.088*** |
| Temperature | 1 | 618.8 | 1332 | 3.019 |
| Flours × conditioning time | 1 | 2081.6 | 2704* | 3.563* |
| Flours × mixing time | 1 | 1016.0 | 169 | 0.035 |
| Flours × washing time | 1 | 1947.0 | 1785 | 0.045 |
| Flours × temperature | 1 | 546.3 | 1463 | 0.975 |
| Conditioning time × mixing time | 1 | 2822.3* | 992 | 0.114 |
| Conditioning time × washing time | 1 | 92.6 | 14 | 2.213 |
| Conditioning time × temperature | 1 | 337.6 | 116 | 5.700** |
| Mixing time × washing time | 1 | 582.0 | 14 | 0.013 |
| Mixing time × temperature | 1 | 61.6 | 33 | 0.001 |
| Washing time × temperature | 1 | 192.5 | 49 | 0.004 |
| Error | 31 | 674.2 | 655 | 0.749 |

* *Indicates 5% level of statistical significance.*

** *Indicates 1% level of statistical significance.*

*** *Indicates 0.1% level of statistical significance.*

† *Since all of the higher order interactions were insignificant, they were omitted from the table.*

residual starch in gluten was considerably affected by the washing time. Although conditioning time had no demonstrable effect when the results were averaged over the entire experiment, a number of significant differential responses involving conditioning time are evident from Table II: these are considered in detail in Table III, together with mean values for all primary factors.

The main feature of these results relates to the low average starch content of the gluten obtained, particularly after the 90 min. washing period: the average starch content was 0.78%. The residual starch content of 2.40% following the 60 min. separation is also highly satisfactory.

The interaction effects of conditioning and dough mixing times on crude gluten weights are described in the data of Table III. Whereas conditioning, on the average, tended to increase the yield of gluten following the three minute dough mixing period, the reverse tendency was noted in conjunction with the nine minute mixing period. The significance of this interaction is probably fortuitous however since it was not exhibited either by starch-free gluten weight or starch content of the gluten (Table II).

TABLE III

MEAN VALUES FOR PRIMARY FACTORS AND SIGNIFICANT DIFFERENTIAL EFFECTS IN THE PRODUCTION OF GLUTEN FROM WHEAT FLOUR

*Primary factors, means of 32 observations*

| Variable under test | Wt. of gluten, moisture-free, gm. | Starch in gluten, % |
|---|---|---|
| Flours | | |
| Straight | 355 | 1.68 |
| Clear | 410 | 1.50 |
| Mixing times, min. | | |
| 3 | 382 | 1.67 |
| 9 | 383 | 1.51 |
| Conditioning times, min. | | |
| 0 | 379 | 1.39 |
| 60 | 386 | 1.78 |
| Washing temperature, ° C. | | |
| 27 | 380 | 1.37 |
| 37 | 386 | 1.81 |
| Washing times, min. | | |
| 60 | 388 | 2.40 |
| 90 | 377 | 0.78 |

*Differential responses, means of 16 observations*

| | | Conditioning time, min. | |
|---|---|---|---|
| | | 0 | 60 |
| Weight of crude gluten, gm. | | | |
| Interaction between conditioning and mixing times | Mixing time, min. | | |
| | 3 | 372 | 392 |
| | 9 | 387 | 380 |
| Weight of starch-free gluten, gm. | | | |
| Interaction between conditioning time and flours | Flour | | |
| | Straight | 353 | 345 |
| | Clear | 395 | 412 |
| Residual starch content of gluten, % | | | |
| Interaction between conditioning time and flours | Flour | | |
| | Straight | 1.25 | 2.11 |
| | Clear | 1.54 | 1.46 |
| Interaction between conditioning time and washing temperature | Washing temp., °C. | | |
| | 27 | 1.48 | 1.27 |
| | 37 | 1.31 | 2.30 |

Average starch-free gluten weights indicate a slight decrease with dough conditioning for the straight flour, whereas a rather marked reverse tendency is noted for the clear flour (Table III).

Table III also shows interaction effects of dough conditioning with flours and with washing temperature upon the residual starch content of gluten. The gluten of straight flour had more residual starch following conditioning;

TABLE IV

EFFECTS OF WASHING TIME, RECIRCULATION OF STARCH SUSPENSION, RATE OF WATER ADDITION, AND AMOUNT OF WATER USED ON RESIDUAL STARCH IN GLUTEN

(All separations at 27° C.)

| Total washing time, min. | Type of separation | Rate of water flow, kgm./min. | | Total wt. of water used for washing, kgm. | Residual starch in gluten, % |
|---|---|---|---|---|---|
| | | During first 15 min. of wash | After first 15 min. | | |
| 45 | Continuous circulation of fresh water | 2 | 1 | 75.0 | 7.3 |
| | Continuous circulation of fresh water | 1 | 1 | 60.0 | 7.9 |
| | Continuous circulation of fresh water | 0.5 | 0.5 | 37.5 | 8.8 |
| | Continuous circulation of fresh water | + | (2(10 min.) (1(25 min.) | 60.0 | 9.7 |
| | Continuous circulation of fresh water | + | (4(10 min.) (1(25 min.) | 80.0 | 6.6 |
| | Recirculation after first 15 min. | 2 | 2 | 45.0 | 7.9 |
| | Recirculation after first 15 min. | 2 | 8 | 45.0 | 8.8 |
| | Same, but fresh water last 15 min. | 2 | (2(15 min.) (1(15 min.) | 60.0 | 7.0 |
| Average for 45-min. separations | | | | | 7.99 |
| 60 | Continuous circulation of fresh water | 2 | 1 | 90.0 | 3.6 |
| | Continuous circulation of fresh water | 1 | 1 | 75.0 | 4.2 |
| | Continuous circulation of fresh water | 0.5 | 0.5 | 45.0 | 5.7 |
| | Continuous circulation of fresh water | + | (2(10 min.) (1(40 min.) | 75.0 | 5.7 |
| | Continuous circulation in fresh water | + | (4(10 min.) (1(40 min.) | 95.0 | 2.4 |
| | Recirculation after first 15 min. | 2 | 2 | 45.0 | 6.6 |
| | Recirculation after first 15 min. | 2 | 8 | 45.0 | 4.2 |
| | Same, but fresh water last 15 min. | 2 | (2(30 min.) (1(15 min.) | 60.0 | 6.1 |
| Average for 60-min. separations | | | | | 4.83 |

+ = *No water added for the first 10 min.; then fresh water as indicated.*

this higher quality gluten might be expected to toughen to a greater extent with conditioning and hence retain more starch. On the average, conditioning reduced the residual starch content when used in conjunction with washing

at 27° C. However, conditioning resulted in appreciable increase in residual starch at the higher washing temperature.

*Series B*

In Table IV are given the results of experiments on washing times, recirculation of starch suspension, rate of water addition, and amount of water used on the residual starch in gluten from separations made at 27° C. On the average, the 60 min. washing period resulted in substantially less residual starch in gluten than did the 45 min. period. Recirculation after the first 15 min. resulted in as thorough washing as in some of the continuous separations using approximately similar amounts of water. Lack of fresh water addition during the first 10 min. had little effect on residual starch content; however, when the subsequent rate of water addition was raised to 4 kgm./min. for 10 min., followed by 1 kgm./min. for 40 min., the lowest residual starch content, namely 2.4%, was obtained.

These results indicate that the starch that remains suspended in the recirculated wash water has little effect upon the residual starch content of the gluten. In subsequent experiments, recirculation of the water from the settling trays with and without removal of suspended starch by centrifugation was compared. The difference in residual starch content of the resulting glutens was negligible. Analysis of the recirculated water also showed that starch removal in the settling tray keeps pace with requirements for efficient gluten washing.

It should be noted that fresh water must be employed at the outset since otherwise the gluten breaks up. The biochemical basis of this effect of recycled washwater appears to be closely allied with the formation of starch sludge fractions, the water soluble pentosan of wheat flour appearing to be the principal causal factor (4).

## Acknowledgments

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# PRODUCTION OF ANTIBACTERIAL SUBSTANCES FROM SULPHITE WASTE LIQUOR BY *PENICILLIUM NOTATUM*[1]

By A. G. Lochhead[2] and F. E. Chase[3]

## Abstract

Sulphite waste liquor, boiled and aerated to remove sulphur dioxide, was found to serve as medium for growth of certain penicillin and notatin producing strains of *Penicillium notatum*, after suitable adjustment of reaction and addition of inorganic nitrogen and phosphorus. Without added organic nutrients, or with supplementary sugar only, the antibacterial activity of the culture fluid is due largely to notatin production. A supplement of corn steep liquor has the effect of increasing penicillin and suppressing notatin formation, so that in the presence of added lactose the culture may assay as high as 30 Oxford units of penicillin per ml.

Utilizing only the sugar present in the sulphite waste liquor one strain of *P. notatum* produced three Oxford units per ml. of culture, while additions of corn steep liquor gave values as high as 11 units, and together with bran, 15 units per ml. Though the results do not suggest the practical use of sulphite waste liquor for penicillin production, where crude culture fluids of highest potency are desired, they suggest the value of further study with micro-organisms capable of making use of energy now going to waste for the production of useful metabolic products.

## Introduction

Sulphite waste liquor, a by-product of the pulp and paper industry, constitutes one of our most important industrial waste products, the successful disposal and utilization of which has been a subject of much investigation. Consisting of approximately 10% of organic matter, of which roughly 60% is lignin and 15% fermentable sugar, the waste liquor, in view of the volume to be disposed of, represents a material of much potential value. On the basis of a content of 1.5% fermentable sugar, it has been estimated (4) that Canadian sulphite mills are discharging about 250,000 tons of sugar per year. In this country a small proportion of the total waste liquor is now utilized by microbiological processes involving the manufacture of industrial alcohol and the production of baker's yeast, while from the lignin fraction industrial chemicals are produced to a relatively small extent.

As discharged, sulphite waste liquor is unsuited to the growth of micro-organisms on account of the presence of free sulphur dioxide and a high acidity. When the liquor is boiled and aerated to remove the sulphur dioxide and the pH suitably adjusted, it will support growth of various bacteria, yeasts, and fungi, preferably after the addition of small percentages of mineral nutrients containing nitrogen and phosphorus. We have found such bacterial species as *Aerobacter oxytocum*, *Bacillus macerans*, and *Clostridium butyricum* capable of active gaseous fermentation of the sugar present in the liquor, while the fungus *Endoconidiophora adiposa* has been found by Adams and

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Ledingham (1) able to decompose about 10% of the lignin fraction. The present report describes exploratory tests to note the suitability of sulphite waste liquor as a medium for growth of various strains of *Penicillium notatum* known to be capable of producing penicillin or notatin (penatin, penicillin B) under favourable conditions.

## Methods

Through the co-operation of the Forest Products Laboratory, Department of Mines and Resources, samples of sulphite waste liquor (*SWL*) were provided, which had been boiled, with aeration, to 60% of the original volume. Additions of nutrients were made as detailed below, and in all cases the pH was adjusted with sodium hydroxide to approximately 5.0. Since the pH value was found to fall on heating, final adjustment was made after sterilization. Tests were made for the most part with 100 ml. medium in 500 ml. Erlenmeyer flasks, inoculated with 1 ml. of an aqueous suspension prepared from one-to-two-week-old cultures of the fungi on modified Czapek-Dox or sulphite liquor agar. Incubation was at 26° C.

For assessing the antibacterial potency of the culture fluids two methods were used, (*a*) the serial dilution method, and (*b*) the cylinder plate method as described for penicillin assay (5). For the former, serial dilutions of the culture fluid were made in the sterile assay medium, nutrient broth, or 1% glucose broth. Tubes were inoculated with a standard loopful of a 24-hr. culture of the test organism in nutrient broth. Nutrient broth assay tubes were inoculated with *Staphylococcus aureus* (Strain *F.D.A.* No. 209), while with glucose broth duplicate series were usually prepared and inoculated respectively with *S. aureus* and *Escherichia coli*. After incubation for 24 hr. at 37° C. tubes were examined for evidence of growth inhibition.

## Experimental Results

Preliminary tests showed much variation in the ability of different strains of *Penicillium* to develop on *SWL*. Though all strains showed good growth in *SWL* diluted to 50% concentration with suitable adjustment of pH and with mineral addenda, pronounced differences were noted in the ability of the moulds to develop in the more concentrated liquor (Table I). Since strains No. 437 and No. 502 were superior in this respect the tests were confined largely to these cultures.

*Effect of Concentration of SWL*

Different percentages of *SWL* were incorporated in a basal medium consisting of sodium nitrate 0.3%, potassium dihydrogen phosphate 0.1%, potassium chloride 0.05%, magnesium sulphate 0.05%, ferrous sulphate 0.001%, and lactose 4%. Four strains of *P. notatum* were compared and the culture fluids assayed after 5 and 10 days. The antibacterial titres after 10 days are shown in Table II. Strain No. 437, which under optimum conditions is inferior to No. 473 in notatin, and to No. 486 in penicillin production, proved more active in the presence of *SWL*. The increased titres noted with

TABLE I

GROWTH OF STRAINS OF *Penicillium notatum* ON SULPHITE WASTE LIQUOR

| Cult. No. | Source | *SWL* 100% | *SWL* conc. × 1.6 | *SWL* agar |
|---|---|---|---|---|
| 437 | S. Bornstein (from Fleming, 1936) | ++++ | ++ | ++++ |
| 443 | E. G. D. Murray (Fleming's strain) | + | Tr. | − |
| 451 | C. Thom (No. 144.5767—Heatley's penicillin strain) | + | Tr. | − |
| 473 | W. Kocholaty (No. *PEN* 2, *P. notatum* Westling 77—penatin strain) | ++ | Tr. | ++ |
| 486 | *N.R.R.L.*[1] (No. 1249.*B*21—'surface' penicillin) | + | Tr. | + |
| 502 | *N.R.R.L.* (No. 832—'submerged' penicillin) | ++++ | ++ | ++ |

[1] *Northern Regional Research Laboratory.*

TABLE II

EFFECT OF SULPHITE WASTE LIQUOR ON ANTIBACTERIAL TITRE
*(Staphylococcus aureus)*

| *P. notatum* strain | Assay medium | *SWL* in medium, % | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 8 | 16 | 40 | 80 | 160[1] |
| 437 | Plain broth | 10 | 250 | 50 | 50 | 0 | 0 |
| | Glucose broth | 250 | 250 | 250 | 1250 | 1250 | 0 |
| 451 | Plain broth | 0 | 10 | 10 | 0 | 10 | 0 |
| | Glucose broth | 0 | 10 | 10 | 0 | 10 | 0 |
| 473 | Plain broth | 0 | 10 | 10 | 0 | 10 | 10 |
| | Glucose broth | 10 | 10 | 50 | 10 | 10 | 10 |
| 486 | Plain broth | 0 | 50 | 50 | 50 | 0 | 0 |
| | Glucose broth | 0 | 250 | 250 | 50 | 50 | 10 |

[1] *SWL conc. to 60% orig. vol.*

glucose broth are considered to be attributable to the production of notatin (penatin, penicillin B), which requires the presence of glucose for the exertion of its antibacterial effect (2).

*Effect of Sugars and Corn Steep Liquor*

The effect of adding glucose, lactose, and sucrose respectively to *SWL*, with and without corn steep liquor (*CSL*), on the antibacterial titre was studied in an experiment using *SWL* diluted to 80% original concentration with water and with the addition of 0.3% sodium nitrate. Sugars were added to bring the total fermentable sugar content to 4.5%, while 5% corn steep liquor was used. Two series of flasks were inoculated with strains No. 437 and No. 486 respectively. Since the latter showed relatively poor growth the data for No. 437 only are given in Table III.

TABLE III

EFFECT OF SUGARS AND CORN STEEP LIQUOR ON PRODUCTION OF ANTIBIOTIC EFFECT IN 80% SULPHITE WASTE LIQUOR
(*P. notatum* strain No. 437)

| Days at 26° C. | Assay med. | Test organism | 80% *SWL* + 0.3% sodium nitrate + addenda | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Glucose | Glucose *CSL* | Lactose | Lactose *CSL* | Sucrose | Sucrose *CSL* |
| | | | pH of culture fluids | | | | | |
| 5 | | | 4.7 | 4.7 | 5.9 | 4.7 | 4.9 | 4.7 |
| 9 | | | 4.2 | 4.7 | 6.9 | 5.2 | 4.3 | 4.5 |
| 14 | | | 4.3 | 6.1 | 5.2 | 7.4 | 4.1 | 6.1 |
| | | | Antibacterial titre—dilution method | | | | | |
| 5 | N.B. | *S. aureus* | 0 | 10 | 0 | 20 | 0 | 20 |
| | G.B. | *S. aureus* | 0 | 10 | 320 | 20 | 20 | — |
| | G.B. | *E. coli* | 0 | 0 | 40 | 0 | 0 | 0 |
| 9 | N.B. | *S. aureus* | 0 | 40 | 0 | 80 | 0 | 80 |
| | G.B. | *S. aureus* | 10 | 80 | 640 | 320 | 10 | 160 |
| | G.B. | *E. coli* | 0 | 0 | 40 | 0 | 0 | 0 |
| 14 | N.B. | *S. aureus* | 0 | 160 | 0 | 320 | 0 | 160 |
| | G.B. | *S. aureus* | 0 | 320 | 2560 | 640 | 10 | 320 |
| | G.B. | *E. coli* | 0 | 0 | >80 | 0 | 0 | 0 |
| | | | Cylinder plate assay—Oxford units per ml. | | | | | |
| 5 | N.A. | *S. aureus* | 0 | 0 | 0 | 1 | 0 | 0 |
| 9 | N.A. | *S. aureus* | 0 | 7 | 0 | 15 | 0 | 7 |
| 14 | N.A. | *S. aureus* | 0 | 19 | 0 | 30 | 0 | 15 |

Under the test conditions, the addition of the supplementary sugar in the form of lactose produced the greatest antibiotic effect. The effect of corn steep liquor, known to stimulate penicillin production, is clearly shown by the plain broth titres and by the cylinder plate assays. At the same time the "notatin effect" or "coli factor", evident in the culture fluid to which lactose was added, was reduced by the addition of corn steep liquor, confirming observations of Waksman and Horning (6) and Kocholaty (3).

*Effect of Various Addenda to SWL (100%)*

In experiments not reported, it was noted that the growth of the moulds and antibacterial potency of the culture fluids were increased by the addition of small amounts of potassium dihydrogen phosphate; consequently a series of tests was made on undiluted *SWL* to which 1% potassium dihydrogen phosphate was added in addition to inorganic nitrogen, but which contained no added sugar.

(1) Table IV shows the effect of adding various solid materials to the culture on the antibacterial titre. Glass wool and cotton wool promoted more rapid development of the mould by providing support for growth. Sawdust depressed growth while bran, in addition to promoting growth, altered the nature of the antibiotic effect by promoting penicillin production and depressing notatin.

TABLE IV

EFFECT OF ADDED MATERIAL ON ANTIBACTERIAL TITRE
(100% *SWL*, *P. notatum* strain No. 437)

| Days | Assay med. | Test organism | *SWL* (100%) + sodium nitrate + potassium dihydrogen phosphate | | | | |
|---|---|---|---|---|---|---|---|
| | | | Control | + Glass wool | + Cotton wool | + Sawdust | + Bran |
| 5 | N.B. | *S. aureus* | 0 | 10 | 10 | 0 | 10 |
| | G.B. | *S. aureus* | 0 | 320 | 160 | 0 | 20 |
| | G.B. | *E. coli* | 0 | 0 | 0 | 0 | 0 |
| 9 | N.B. | *S. aureus* | 0 | 0 | 0 | 0 | 20 |
| | G.B. | *S. aureus* | 320 | 1280 | 640 | 20 | 320 |
| | G.B. | *E. coli* | 0 | 160 | 0 | 0 | 0 |
| 14 | N.B. | *S. aureus* | 10 | 0 | 0 | 10 | 40 |
| | G.B. | *S. aureus* | 1280 | 1280 | 1280 | 320 | 320 |
| | G.B. | *E. coli* | 40 | 320 | 320 | 40 | 0 |

(2) The effect of further modification is shown in Table V in which the antibacterial potency of the culture fluid is expressed in Oxford units as determined by the penicillin assay method (5) with values calculated from a penicillin standard curve. The favourable effect of corn steep liquor is brought out, as well as that of bran, the latter showing indications of a 'preservative' effect on the penicillin formed.

TABLE V

EFFECT OF VARIOUS ADDENDA ON PENICILLIN FORMATION IN SULPHITE WASTE LIQUOR (100%)
(*P. notatum*, strain No. 437)

| Medium | pH | Oxford units per ml. | | | | |
|---|---|---|---|---|---|---|
| | | Days | | | | |
| | | 3 | 6 | 9 | 12 | 16 |
| Control—*SWL* (+$NaNO_3$ and $KH_2PO_4$) | 5.1 | 0.3 | 1.1 | 0.8 | 0.2 | 0 |
| *SWL* + 8%*CSL* | 5.0 | 0.5 | 11.0 | 2.0 | 0 | 0 |
| *SWL* + bran | 5.1 | 1.5 | 4.0 | 5.0 | 2.8 | 1.2 |
| *SWL* + straw | 5.1 | 0.6 | 1.2 | 0.7 | 0.3 | 0 |
| *SWL* + *CSL* + bran | 5.0 | 0.9 | 13.0 | 15.0 | 1.3 | 0 |
| *SWL* + *CSL* + straw | 4.9 | 1.2 | 9.0 | 1.2 | 0 | 0 |

*Surface vs. Submerged Cultures*

To compare penicillin production in *SWL* (100%) by surface and submerged growth of the mould two series of tests were made in which two strains of *P. notatum* were used, No. 437 and No. 502, the latter used for commercial scale production of penicillin by the submerged, or tank, method.

Much better growth of the moulds, both in surface and submerged (shaken) cultures, was obtained with sodium nitrate than with ammonium sulphate (Table VI). In *SWL* without supplement other than inorganic nitrogen and phosphorus, a maximum of three units per ml. was obtained. The addition of sugars alone was without effect, though under the test conditions *CSL* increased penicillin formation to eight units per ml., with production generally somewhat better under submerged conditions.

TABLE VI

EFFECT OF VARIOUS ADDENDA ON PENICILLIN FORMATION IN SULPHITE WASTE LIQUOR (100%)
(Surface and submerged cultures)

| *P. notatum* strain | Culture medium | pH | Oxford units per ml. | | | |
|---|---|---|---|---|---|---|
| | | | Surface | | Submerged | |
| | | | 6 Days | 10 Days | 6 Days | 10 Days |
| | *SWL* + 0.1% $KH_2PO_4$ | | | | | |
| 437 | + 0.3% $NaNO_3$ | 5.1 | 1.0 | 0.7 | 0.4 | 0.8 |
| | + 0.3% $(NH_4)_2SO_4$ | 5.1 | 0 | 0.3 | 0 | 0.2 |
| 502 | + 0.3% $NaNO_3$ | 5.1 | 0.8 | 0.8 | 1.3 | 3.0 |
| | + 0.3% $(NH_4)_2SO_4$ | 5.1 | 0 | 0.3 | 0 | 0.5 |
| | | | 4 Days | 9 Days | 4 Days | 7 Days |
| | *SWL* + 0.1% $KH_2PO_4$ + 0.3% $NaNO_3$ | | | | | |
| 437 | Control | 5.0 | 0.6 | 0.8 | 0 | 1.3 |
| | + 1% glucose | 5.0 | 0.7 | 1.0 | 0 | 0 |
| | + 1% lactose | 5.0 | 0.5 | 0.8 | 0.2 | 0.3 |
| | + 8% *CSL* | 5.0 | 2.2 | 7.0 | 4.0 | 8.0 |
| | + 20% whey | 5.1 | 1.9 | 2.0 | 0.3 | 0.2 |
| 502 | Control | 5.0 | 0.8 | 2.2 | 0 | 2.0 |
| | + 1% glucose | 5.0 | 1.2 | 1.2 | 0 | 0.2 |
| | + 1% lactose | 5.0 | 0.6 | 1.9 | 0.3 | 0.4 |
| | + 8% *CSL* | 5.0 | 4.0 | 8.0 | 6.5 | 6.5 |

## Discussion

The data indicate that some strains of *Penicillium notatum* are able to develop in *SWL* with production of antibacterial substances to a degree varying with the nature of the added nutrients or growth-stimulating factors. Without added organic nutrients, or with supplementary sugar only, the antibacterial activity of the culture fluid is due principally to notatin (Tables III and IV). A supplement of corn steep liquor has the effect of increasing penicillin, and

suppressing notatin formation so that, in the presence of added lactose, the culture fluid may assay as high as 30 units penicillin per ml.

With no modification of *SWL* other than adjustment of pH and the addition of inorganic nitrogen and phosphorus, one penicillin producing strain of *P. notatum* was able to form penicillin up to three Oxford units per ml. culture fluid, depending thus upon the sugar of the *SWL* (approx. 1.5%) as source of energy. Additions of corn steep liquor gave values as high as 11 units, and together with bran, 15 units per ml. culture fluid (Tables V and VI). Such values are considerably less than those obtained by use of the most appropriate substrates, as used for commercial penicillin production, and do not suggest the practical use of *SWL* under present conditions where cost of raw material must be weighed against the need for producing crude culture fluids of highest potency. The findings, however, suggest the value of more extensive study, not only with *P. notatum* but with other micro-organisms that may be capable of making use of energy, now going to waste in *SWL*, for the elaboration of antibiotic substances or other metabolic products.

## Acknowledgment

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# SMOKED MEATS

## IV. MEASUREMENT OF COLOUR AND COLOUR STABILITY OF WILTSHIRE BACON[1]

By Jesse A. Pearce[2] and A. H. Woodcock[3]


### Abstract

Colour and colour stability of smoked and unsmoked Wiltshire bacon stored for periods up to 98 days at −18°, −9°, −1°, and 7° C. were measured by nine- and by three-filter methods. Differences in brightness between bacon sides were appreciable. Decrease in brightness with time was evident after 70 days at 7° C., but not until after 98 days at freezer temperatures. Smoking increased brightness.

There were marked differences in colour quality between bacon sides. Smoking decreased the proportion of red colour in the meat with corresponding increases in blue and green. Colour stability measurements on all samples showed that exposure caused a decrease in the proportion of red and an increase in green and green-blue.


### Introduction

During the course of preliminary work on smoked meats it was noted that the colour of smoked meat was lighter and less stable on storage than that of unsmoked meat (3). A more comprehensive experiment was undertaken, primarily to assess the chemical changes occurring in smoked meat (2) but the opportunity was taken to record the colour changes as well. These were measured by two methods developed in these laboratories: the earlier method was based on the use of the three primary colour bands, red, green, and blue (4); the later method consisted of the more elaborate nine-filter method (5), designed to give more precise information.

### Materials

It was known that bacon was of a highly variable nature. Therefore the experimental design permitted a comparison to be made between smoked and unsmoked portions of the same side of bacon, since smoking was the primary factor under consideration.

The material used was fully described elsewhere (2), and treatment may be outlined briefly as follows: the right and left sides of four hogs were cured, allowed to drain and partially mature for five days at 3.3° C. (38° F.), and the back and gammon removed from each side. After allotting two backs at random to each of the storage temperatures of −18°, −9°, −1°, and 7° C., (0°, 15°, 30°, and 45° F.) each back was divided into two approximately equal portions, one of which was smoked at an air temperature of approximately 60° C. (140° F.) for 14 hr.

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*Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as Paper No. 128 of the Canadian Committee on Food Preservation and as N.R.C. No. 1269.*

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## Methods

Brightness (intensity) and colour quality measurements were made by a three-filter (4) and by a nine-filter method (5) used in this laboratory. In addition colour stability measurements (1) were made on samples exposed for 72 hr. at 7° C. and 95% relative humidity.

The nine-colour method (5) was designed primarily to measure colour quality, brightness of the sample relative to the standard white block being determined as an item separate from analysis of colour quality. By this method, chroma or colour quality was determined as light scattered by the sample within any one of the nine colour bands. It is expressed as a fraction of the total light scattered by the sample in relation to the light scattered by the standard white surface (magnesium carbonate). As has been previously pointed out (5), the analytical work can be reduced by selecting the most informative of the nine colour regions, appropriate to the experimental material.

The three-filter method (4) was designed and operated to distinguish a combination of brightness and colour quality in three regions of the spectrum (red, green, and blue), the colour value being determined from the scatter by the surface under investigation as a percentage of the colour scattered by the surface of the standard white block when both surfaces were illuminated by the same source. Brightness was considered to be the sum of the light scattered in the three colour regions.

Since it has been pointed out (5) that the colour quality data using the three-filter method were closely correlated with brightness data using the nine-filter method, an attempt was made to eliminate brightness effects in the three-filter method. This was done by taking the ratio of colour value by the three-filter method (Table III) to brightness by the nine-filter method.

## Results

The small differences in the results of colour measurements on meat necessitated statistical treatment of the data (1, 3). For purposes of statistical analyses (2) results at 7° C. (chill temperature) could not be included with results at −1°, −9°, and −18° C. (freezer temperatures). Therefore results at chill temperature are shown by graphs of mean values at the chill temperature in relation to graphs of the average results for all freezer temperatures (Figs. 1 and 2).

The general lack of statistically significant differences that could be attributed to temperature effects, shown in Tables II and IV, is accounted for by the fact that differences between the sides of bacon were relatively enormous. There was no instance of parallel behaviour of any two sides at the same temperature. Although the statistical design permitted demonstration of significant storage effects, in most instances the inherent differences between sides were as great or greater than differences attributable to changes during storage. Thus, variability in the product was greater than any differences due to storage temperatures or times.

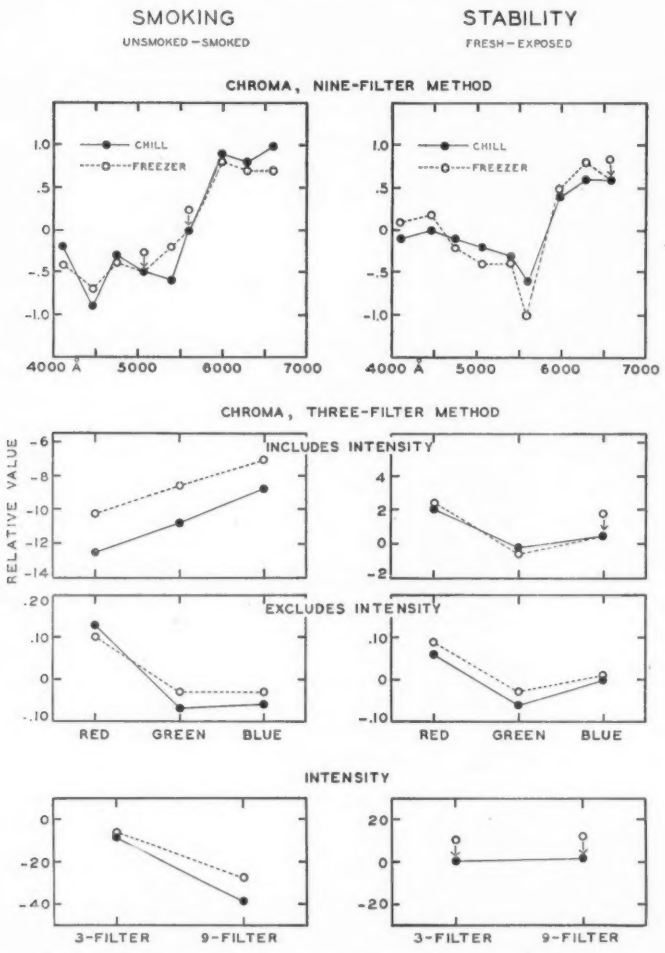


FIG. 1. *Relative differences resulting from the effect of smoking and stability tests on sides stored at chill and freezer temperatures.*

*Brightness or Intensity*

Measurements by the nine-filter method on samples stored at freezer temperatures showed that smoking increased and storage time decreased brightness, but exposure had no significant effect (Tables I and II). The only differential effect having practical significance was the result of a general increase in brightness of samples stored at −18° and −9° C. up to the 70-day sampling followed by a pronounced decrease at the 98-day sampling, while at −1° C., a regular decrease occurred throughout the storage period. Smoked sides stored at the chill temperature had greater brightness than sides stored

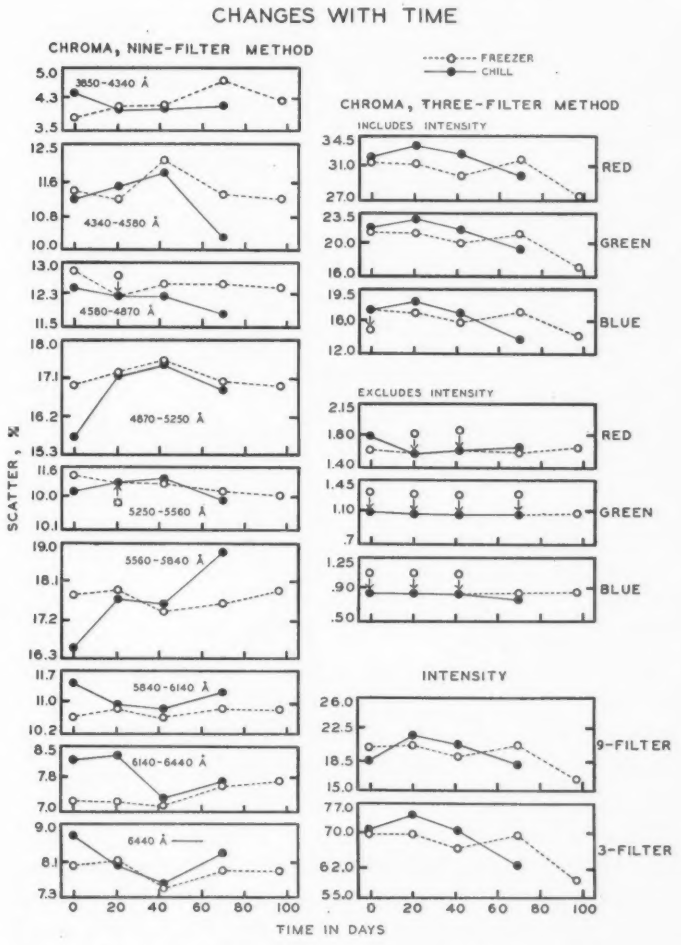


FIG. 2. *The effect of time on colour factors of meats stored at chill and freezer temperatures.*

at the other temperatures (Fig. 1), but brightness decrease with time was slightly greater (Fig. 2). Exposure again had only a minor effect on brightness.

Smoking, time, and exposure were observed to affect total brightness of samples held at freezer temperatures as measured by the three-filter method (Tables III and IV), the important difference from the nine-filter data being the significant decrease in brightness resulting from exposure. This might have been expected since the three-filter method gives an accumulative brightness effect, thereby amplifying any existing small differences. Smoking caused a greater increase of total brightness in sides stored at 7° C., and change on exposure was no different from that occurring in materials stored at freezer

TABLE I

TABLE OF MEANS FOR INTENSITY AND COLOUR QUALITY MEASUREMENTS BY THE NINE-FILTER METHOD ON SMOKED AND UNSMOKED WILTSHIRE BACON SUBJECTED TO STORAGE TEMPERATURES OF −18°, −9°, AND −1° C. AND TO A STABILITY TEST AT 7° C. AND 95% RELATIVE HUMIDITY

| Variable under investigation | Intensity or brightness | Chroma or colour quality | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Blue | | | Green | | | Red | | |
| | | 3850–4340Å | 4340–4580Å | 4580–4870Å | 4870–5250Å | 5250–5560Å | 5560–5840Å | 5840–6140Å | 6140–6440Å | Above 6440Å |
| Temperature (°C.) | | | | | | | | | | |
| −18 | 18.7 | 4.3 | 11.2 | 12.4 | 17.1 | 11.1 | 17.8 | 10.7 | 7.4 | 8.0 |
| − 9 | 19.7 | 4.0 | 11.5 | 12.5 | 17.2 | 11.2 | 17.8 | 10.7 | 7.3 | 7.8 |
| − 1 | 19.1 | 4.2 | 11.6 | 12.4 | 17.1 | 11.1 | 17.5 | 10.8 | 7.4 | 7.9 |
| Smoking | | | | | | | | | | |
| Unsmoked | 15.3 | 4.0 | 11.1 | 12.2 | 16.9 | 11.0 | 17.7 | 11.1 | 7.7 | 8.2 |
| Smoked | 23.0 | 4.4 | 11.8 | 12.6 | 17.4 | 11.2 | 17.7 | 10.3 | 7.0 | 7.5 |
| Time (days) | | | | | | | | | | |
| Initial | 20.0 | 3.8 | 11.4 | 12.8 | 16.9 | 11.4 | 17.8 | 10.6 | 7.2 | 8.0 |
| 21 | 20.3 | 4.1 | 11.2 | 12.2 | 17.2 | 11.3 | 17.9 | 10.8 | 7.2 | 8.1 |
| 42 | 19.0 | 4.1 | 12.1 | 12.5 | 17.5 | 11.2 | 17.4 | 10.6 | 7.1 | 7.5 |
| 70 | 20.2 | 4.7 | 11.3 | 12.5 | 17.0 | 11.0 | 17.6 | 10.8 | 7.6 | 7.9 |
| 98 | 16.4 | 4.2 | 11.2 | 12.4 | 16.9 | 10.9 | 17.9 | 10.8 | 7.7 | 7.9 |
| Stability | | | | | | | | | | |
| On removal from storage | 19.3 | 4.2 | 11.5 | 12.3 | 16.9 | 11.0 | 17.2 | 11.0 | 7.8 | 8.2 |
| After exposure | 19.1 | 4.1 | 11.3 | 12.5 | 17.3 | 11.4 | 18.2 | 10.5 | 7.0 | 7.6 |

temperatures (Fig. 1). Decrease in brightness with time was evident after 70 days at 7° C. but not until the 98th day sampling at freezer temperatures (Fig. 2).

*Colour Quality—Nine-filter Method*

Smoking, time of storage, and exposure all caused significant changes in colour quality of samples stored at freezer temperatures (Tables I and II). Smoking increased the proportion of blue and blue-green in the samples and decreased the proportion of red. Differences in colour quality as storage progressed were irregular and in general no marked change occurred. There seems to be some evidence of a maximum or a minimum occurring at about the 42-day sampling (Fig. 2). Exposure after storage caused a general decrease in the proportion of red and an increase in green and green-blue. Sides stored at −9° C. were much less susceptible to change on exposure.

The smoked sides used at the chill temperature had a greater proportion of blue and a smaller proportion of red (Fig. 1). Changes in colour quality with time (Fig. 2) again indicated a tendency for a maximum or a minimum to occur at the 42-day sampling. Stability tests indicated that less drastic colour changes occurred after chill storage (Fig. 1). This might again be

TABLE II

ANALYSIS OF VARIANCE OF INTENSITY AND COLOUR QUALITY MEASUREMENTS BY THE NINE-FILTER METHOD ON SMOKED AND UNSMOKED WILTSHIRE BACON SUBJECTED TO STORAGE TEMPERATURES OF −18°, −9°, AND −1° C., AND TO A STABILITY TEST AT 7° C. AND 95% RELATIVE HUMIDITY

| Source of variance | Degrees of dom | Mean square | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Intensity or brightness | Chroma or colour quality | | | | | | | | |
| | | | Blue | | | Green | | | Red | | |
| | | | 3850–4340Å | 4340–4580Å | 4580–4870Å | 4870–5250Å | 5250–5560Å | 5560–5840Å | 5840–6140Å | 6410–6440Å | Above 6440Å |
| Temperature | 2 | 9.2 | 0.90* | 2.1 | 0.12 | 0.06 | 0.20 | 1.4 | 0.22 | 0.29 | 0.44 |
| Between sides within temperature (Error I) | 3 | 135 | 0.38 | 1.4 | 0.32 | 2.1 | 0.88 | 2.5 | 0.86 | 1.0 | 4.8 |
| Smoking | 1 | 1785** | 4.3** | 14.0** | 4.1** | 6.9* | 4.1** | 0.00 | 18** | 15** | 17* |
| Smoking × temperature | 2 | 89 | 0.37 | 2.9** | 0.49* | 0.79 | 0.32 | 0.06 | 0.29 | 0.73* | 2.9 |
| Smoking × sides within temp. (Error II) | 3 | 36 | 0.09 | 0.52 | 0.15 | 0.49 | 0.40 | 0.58 | 0.58 | 0.27 | 0.77 |
| Time | 4 | 65** | 2.4** | 3.1** | 1.8** | 1.6** | 1.1** | 1.2* | 0.31 | 1.8** | 1.3* |
| Time × temp. | 8 | 18* | 0.21 | 0.35 | 0.13 | 0.60** | 0.28 | 0.22 | 0.36* | 0.74** | 1.6** |
| Time × smoking | 4 | 17 | 0.16 | 0.37 | 0.51** | 0.05 | 0.05 | 1.0 | 0.26 | 0.44 | 0.56 |
| Residual (Error III) | 24 | 6.6 | 0.19 | 0.21 | 0.16 | 0.17 | 0.14 | 0.38 | 0.14 | 0.26 | 0.45 |
| Stability | 1 | 1.0 | 0.24 | 1.1* | 1.5** | 4.0** | 5.3** | 33** | 7.4** | 18** | 11** |
| Stability × temp. | 2 | 0.16 | 0.25 | 0.12 | 0.16 | 0.77** | 0.64** | 1.9** | 0.20 | 1.3** | 0.38** |
| Stability × smoking | 1 | 0.03 | 0.32 | 0.24 | 0.01 | 0.18 | 0.04 | 0.38 | 0.11 | 0.07 | 0.00 |
| Stability × time | 4 | 0.81 | *1.6** | 0.30 | 0.63** | 0.11 | 0.16 | 0.12 | 0.19 | 0.50 | 0.41 |
| Residual (Error IV) | 30 | 0.25 | 0.21 | 0.25 | 0.10 | 0.09 | 0.07 | 0.21 | 0.09 | 0.18 | 0.06 |
| Machine variation | 26 | | 0.19 | 0.16 | 0.08 | 0.14 | 0.05 | 0.09 | 0.02 | 0.02 | 0.18 |

* *Exceeds 5% level of statistical significance.*
** *Exceeds 1% level of statistical significance.*

attributed to differences between sides, combined with the possibility that greater changes had occurred during storage at chill than occurred at freezer temperatures and therefore less change took place on exposure.

*Colour Quality—Three-filter Method*

The effect of smoking on samples stored at freezer temperatures appeared to be an increase in all three colours: this, however, was the result of the overall increase in brightness since exclusion of brightness effects showed behaviour similar to that observed by the nine-filter method, i.e. a decrease in red and an increase in green and blue (Tables III and IV). This seemed to indicate that the proportion of red colour in smoked meats was smaller than in unsmoked meats although visually it appeared greater because of the brightness effects.

TABLE III

TABLE OF MEANS FOR INTENSITY AND COLOUR MEASUREMENTS BY THE THREE-FILTER METHOD ON SMOKED AND UNSMOKED WILTSHIRE BACON SUBJECTED TO STORAGE TEMPERATURES OF −18°, −9°, AND −1° C. AND TO A STABILITY TEST AT 7° C. AND 95% RELATIVE HUMIDITY

| Variable under investigation | Total brightness | Chroma or colour quality | | | | | |
|---|---|---|---|---|---|---|---|
| | | Including intensity | | | Excluding intensity | | |
| | | Blue | Green | Red | Blue | Green | Red |
| Temperature (°C.) | | | | | | | |
| −18 | 65.5 | 15.8 | 20.0 | 29.7 | 0.85 | 1.06 | 1.61 |
| − 9 | 67.8 | 16.3 | 20.6 | 31.0 | 0.84 | 1.04 | 1.59 |
| − 1 | 66.8 | 16.0 | 20.0 | 30.2 | 0.84 | 1.04 | 1.60 |
| Smoking | | | | | | | |
| Unsmoked | 54.0 | 12.5 | 15.9 | 25.2 | 0.82 | 1.03 | 1.65 |
| Smoked | 79.6 | 19.5 | 24.5 | 35.4 | 0.85 | 1.06 | 1.55 |
| Time (days) | | | | | | | |
| Initial | 69.9 | 17.0 | 21.2 | 31.5 | 0.84 | 1.05 | 1.60 |
| 21 | 69.5 | 16.8 | 21.1 | 31.2 | 0.83 | 1.03 | 1.55 |
| 42 | 66.2 | 15.6 | 19.9 | 29.9 | 0.82 | 1.02 | 1.58 |
| 70 | 69.3 | 16.8 | 21.0 | 31.6 | 0.83 | 1.04 | 1.58 |
| 98 | 59.0 | 14.0 | 17.0 | 27.2 | 0.85 | 1.05 | 1.64 |
| Stability | | | | | | | |
| On removal from storage | 67.8 | 16.3 | 19.9 | 31.4 | 0.84 | 1.03 | 1.65 |
| After exposure | 65.5 | 15.8 | 20.5 | 29.1 | 0.83 | 1.06 | 1.54 |

Changes in colour quality resulting from time of storage at freezer temperatures corresponded closely with the changes shown to occur by brightness measurements. However, elimination of brightness effects indicated that in general storage time had little effect on the amount of any one of these three colours in the meat.

Colour quality measurements (including brightness) showed that decreases in red and blue and increases in green scatter resulted from exposure after storage. After excluding brightness only a decrease in red and increase in green was apparent. These latter results agreed with observations by the nine-filter method.

Colour quality values (excluding brightness) on samples stored at 7° C. showed changes attributable to smoking and exposure similar to those measured by the nine-filter method (Fig. 1). Time changes at the freezer temperatures measured by the three-filter method were largely attributable to changes in brightness; excluding brightness gave results similar to those occurring at the chill temperatures (Fig. 2).

## Discussion

While the results by the nine-filter method have not been grouped as suggested (5) it is evident that the greatest number of significant changes

TABLE IV

ANALYSIS OF VARIANCE OF INTENSITY AND COLOUR QUALITY MEASUREMENTS BY THE THREE-FILTER METHOD ON SMOKED AND UNSMOKED WILTSHIRE BACON SUBJECTED TO STORAGE TEMPERATURES OF −18°, −9°, AND −1° C., AND TO A STABILITY TEST AT 7° C. AND 95% RELATIVE HUMIDITY

| Source of variance | Degrees of freedom | Mean square | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Total brightness | Chroma or colour quality | | | | | |
| | | | Including intensity | | | Excluding intensity | | |
| | | | Blue | Green | Red | Blue | Green | Red |
| Temperature | 2 | 59 | 3.5 | 4.5 | 16 | 0.0004 | 0.0078 | 0.0060 |
| Between sides within temperature (Error I) | 3 | 1369 | 101 | 163 | 201 | 0.0004 | 0.0002 | 0.0358 |
| Smoking | 1 | 19,630** | 1478** | 2205** | 3154** | 0.0323** | 0.0163* | 0.3070* |
| Smoking × temperature | 2 | 1066 | 81 | 129 | 155 | 0.0016 | 0.0056 | 0.0280 |
| Smoking × sides with temperature (Error II) | 3 | 411 | 28 | 54 | 60 | 0.0012 | 0.0067 | 0.0119 |
| Time | 4 | 490** | 39** | 49* | 83** | 0.0032 | 0.0149* | 0.0540** |
| Time × temperature | 8 | 224* | 18* | 27 | 35 | 0.0017 | 0.0015 | 0.0045 |
| Time × smoking | 4 | 184 | 14 | 14 | 28 | 0.0002 | 0.0008 | 0.0038 |
| Residual (Error III) | 24 | 98 | 7.2 | 12 | 16 | 0.0021 | 0.0036 | 0.0057 |
| Stability | 1 | 158** | 6.3** | 11** | 157** | 0.0082 | 0.0347** | 0.3532** |
| Stability × temperature | 2 | 31** | 1.3 | 0.45 | 20** | 0.0028 | 0.0008 | 0.0638** |
| Stability × smoking | 1 | 11 | 5.4** | 1.0 | 9.9** | 0.0078 | 0.0028 | 0.0006 |
| Stability × time | 4 | 4.7 | 1.4* | 1.1 | 2.9* | 0.0044 | 0.0012 | 0.0222** |
| Residual (Error IV) | 30 | 4.1 | 0.50 | 1.5 | 0.80 | 0.0021 | 0.0029 | 0.0034 |

* *Exceeds 5% level of statistical significance.*
** *Exceeds 1% level of statistical significance.*

occurred in bands 4580–4870Å and 6140–6440Å. The first of these bands is considered to be a characteristic absorption band of nitrosohaemoglobin and the second should represent changes in methaemoglobin (5). The results of measurements in these two colour bands can be interpreted in terms of changes in these components.

Temperature effects were not significant and time effects were extremely variable. However smoking and exposure both appeared to increase the nitrosohaemoglobin and to decrease the methaemoglobin. Interpretation of these results, in terms of changes in components, must, however, be treated with some reserve, since the method of making the measurement is dependent upon the ratio of the amount of light scattered in the various bands. Therefore, an increase in the light scattered in any one band must result in a decrease of light scattered in one or more of the other bands.

With respect to the measurement of colour attributes of meat, the foregoing indicates that the observations by either three- or nine-filter methods were satisfactory. The three-filter method required adjustment of the colour quality values to eliminate confusing colour quality with brightness. The nine-filter method permitted a more precise differentiation of colour quality, but in general the same conclusions can be drawn from results by either method.

## Acknowledgments

The authors wish to thank Mr. W. D. B. Reid, National Research Laboratories, for making the statistical computations.

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# DRIED MILK POWDER

## I. METHODS OF ASSESSING QUALITY AND SOME EFFECTS OF HEAT TREATMENT[1]

By Jesse A. Pearce[2]


## Abstract

The suitability of a number of objective tests of milk powder quality was assessed against subjective scores of palatability. The objective tests investigated were: oxygen and water sorption of the powders; chlorophyll and peroxide oxygen values of the fat; 'browning' of the powder; fluorescence values; changes in peroxidase, trimethylamine, volatile sulphur compounds, and diacetyl content; solubility by centrifuging and a potassium chloride solution method; titratable acidity; pH; congo rubin and iron numbers; foaming volume; coagulation by acid, alcohol, and rennet; dielectric constant; colour intensity and colour quality; refractive index; viscosity and surface tension. The subjective measurement of palatability was finally adopted as the most precise measure of milk powder quality.

While measurement of peroxidase activity was unsatisfactory in the determination of quality, the activity of this enzyme was observed to decrease with increase in time and temperature.

When palatability was used as a measure of quality, powders stored at 37.8° C. for seven days were preferred to powders stored at 26.7°, 48.9°, or 60.0° C. Interpretation of these results in terms of the temperature to which milk powder should be cooled indicated that 37.8° was the desirable temperature. Current commercial practice permits cooling to this temperature within a few minutes after the completion of drying.


## Introduction

For a number of years it has been considered advisable to cool milk powders to room temperature immediately on completion of drying (13, p. 524). Drum-dried powders are believed to cool rapidly to this temperature, while spray-dried powders are often left in the drier for long periods, or packed without cooling. Spray-dried powders packed without cooling have been observed to have temperatures of 57° to 60° C. after 24 to 48 hr. (13, p. 521). However, no published information was found to indicate just how much deterioration may occur, or to what temperature it is desirable to cool the powder.

Data on dried whole egg powder show that the powder should be cooled to a temperature of 26.7° C. within three hours of its preparation if deterioration is to be prevented (21). The present paper is primarily concerned with the results of a similar experiment on spray-dried milk powder. However, before proceeding with the investigation some consideration was given to methods of measuring milk powder quality. Observations on these measurements are also recorded.

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## Tests of Milk Powder Quality

Subjective measurements of quality are generally affected by the interest and physical condition of the individual and even a large panel may vary in its average opinion from day to day. While individual tasters with great experience may apply reasonably uniform scores to food products that are tasted or smelled, their results may not conform closely to the opinion of the general public. Therefore, an objective measure of quality is to be preferred. To be satisfactory this objective measure must correlate closely with tasters' opinions. Since a great number of tests of milk powder quality are being used by various investigators, it was believed desirable to make a comprehensive survey of existing tests and to study other possible measures of quality. Existing tests have generally been used in studies where probably only one factor of milk powder deterioration was involved. Therefore these tests were evaluated on samples subjected to deterioration from several possible causes.

*Material*

Samples were obtained from six producers of spray-dried whole milk powder. From three of these, powder samples were supplied in August, September, October, 1943. The samples were divided into subsamples, some of which were tested without further treatment while others were assigned at random to a variety of storage conditions. These conditions were arranged to give different time of exposure to different temperatures at about 14° C. (25° F.) intervals from 0° to 60° C. (32° F. to 140°); to relative humidities of 16 and 85%; to atmospheres of air, nitrogen, and carbon dioxide; and to bright sunlight. Total number of samples tested was 38.

*Methods Used for Testing Powder Quality*

Two variations of the subjective method were used. One taste panel of 14 people scored the powders on the basis of 10 to 0, 10 being the equivalent of excellent fresh whole milk. The other panel, consisting of 10 people, scored on a basis of 3 to 0, 3 being the equivalent of excellent fresh whole milk.

The method of reconstituting milk powder was the same for both panels; 10 gm. of milk powder was mixed for 30 sec. in 80 ml. of distilled water at 38° C. (100° F.) in a Waring blendor and then chilled to 7° C. (45° F.) (11).

The objective tests included measurements of oxygen (10) and water sorption (15) of the powders. Changes in the fat were measured by determination of the chlorophyll value (4) and a colorimetric method for the determination of peroxide (3). The latter was chosen in preference to the iodometric method (16) commonly used since marked deteriorations occurred in milk fat before measurable values developed (18). Colorimetric measurements to detect 'browning' in the powder (7) and variations of the fluorescence measurement previously used (18) were also made. Changes in some components were measured by tests for peroxidase (6), trimethylamine (2), volatile sulphur-containing substances (19), and a test for diacetyl based on the oxidation of this component to acetic acid by hydrogen peroxide. Solubility was deter-

mined by a recommended method (1) and by the potassium chloride solution method used for dried whole egg powder (20). Titratable acidity (1) and pH were also determined. Colloidal characteristics, congo rubin numbers (12, pp. 58–63) and an iron number (9, p. 193), foaming volume (8), and coagulation by alcohol, by acid, and by rennet were also studied. Rennet coagulation was measured as the 'time of set' required for the sol–gel transformation of the reconstituted milk and rennet; time of set was determined by a 'tilted rod' method (14). Measurements were made of such physical characteristics as dielectric constant in a high viscosity, low dielectric oil (17, pp. 208-216), colour intensity and colour quality (22), refractive index, viscosity, and surface tension.

*Results*

Objective tests have been found by other investigators to be reasonably successful when the material was subjected to a single factor affecting quality. In the present work, it was believed desirable to test many factors simultaneously; under these conditions all the objective tests studied were unsuitable. Two of the tests, oxygen and water sorption, were found to be too cumbersome for routine control work and were omitted after a few trials. No method was found that would give an extract sufficiently clear for satisfactory refractive index measurements. With many of the tests, e.g. chlorophyll value, congo rubin, and iron numbers and coagulation by alcohol and acid, it was not possible to obtain a satisfactory end-point. Measurements of trimethylamine, of volatile sulphur-containing compounds, of coagulation by rennet, and of foaming volume were not easily duplicated. For many other tests, e.g. solubility by the potassium chloride solution method, pH, viscosity, surface tension, and dielectric constant, the error of duplication was equal to, or greater than, the difference due to quality.

Correlations of palatability with the remaining objective tests are shown in Table I; titratable acidity was significantly related to palatability although it was not a satisfactory method of predicting eating quality.

TABLE I

CORRELATIONS BETWEEN VARIOUS QUALITY TESTS ON SPRAY-DRIED WHOLE MILK POWDER

| Palatability, Panel I correlated with: | Correlation coefficient (36 D.f.) |
|---|---|
| Peroxidase value (5) | 0 |
| Colour intensity (22) | .06 |
| Peroxide values (3) | −.11 |
| Solubility index (1) | −.18 |
| Colorimetric value (7) | −.20 |
| Diacetyl value | −.23 |
| Fluorescence (18) | −.26 |
| Titratable acidity (1) | −.47** |
| Palatability, Panel II | .79** |

** *Exceeds 1% level of statistical significance.*

It was possible that the palatability determinations lacked accuracy hence the testing by two different panels, scoring on two different scales. The correlation, shown graphically in Fig. 1, was reasonably close (Table I), although an error of about 35% was attributable to the tasters' judgments. Nevertheless, organoleptic determination of quality was a more precise measure than any objective test. Therefore, it was believed desirable to proceed with the investigation using the subjective test. However investigations of objective methods of measuring milk powder quality are continuing.

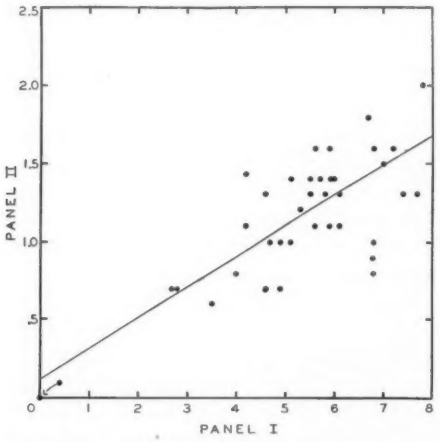


FIG. 1. *Relation between the taste scores given by two panels to dried milk powders of varying quality.*

## Effect of Heat-Treatment on Quality

While palatability scores by both panels (Table II) were considered the primary criteria of quality in this investigation, one of the other tests, the peroxidase measure, gave results believed worthy of some consideration. These results are also recorded (Fig. 2).

*Materials and Methods*

Spray-dried skim milk powder (1% fat), and whole milk powder (28% fat), prepared by a commercial processor, and an experimental lot of spray-dried whole milk powder (28% fat) were stored at 26.7°, 37.8°, 48.9, and 60° C. (80°, 100°, 120°, and 140° F.) for seven days. The experimental powder was prepared using pasteurizing, condensing, and drying temperatures about 11° C. (20° F.) lower than those used by the commercial processor since it was felt that currently used temperatures may be too high to give an excellent quality of product. Drying was done on a laboratory spray-drier (24).

Skim milk was reconstituted by mixing 10 gm. of powder with 100 ml. of distilled water and a palatability reference score of 10 indicated an equivalence to excellent fresh skim milk.

TABLE II

TABLE OF MEAN VALUES AND ANALYSIS OF VARIANCE OF SPRAY-DRIED MILK POWDERS SUBJECTED TO 26.7°, 37.8°, 48.9°, AND 60.0° C. FOR SEVEN DAYS

*Table of mean values*

| Variable under study | Palatability score | |
|---|---|---|
| | Panel I | Panel II |
| Samples | | |
| Skim milk powder (commercial) | 4.0 | 0.6 |
| Whole milk powder (commercial) | 5.8 | 1.4 |
| Whole milk powder (experimental) | 6.0 | 1.5 |
| Temperature (°C.) | | |
| 60.0 | 5.0 | 0.9 |
| 48.9 | 5.5 | 1.1 |
| 37.8 | 5.5 | 1.4 |
| 26.7 | 5.2 | 1.3 |
| Time (days) | | |
| Initial | 5.3 | 0.7 |
| 1 | 5.0 | 1.2 |
| 2 | 5.6 | 1.0 |
| 3 | 5.1 | 1.0 |
| 4 | 5.2 | 1.3 |
| 5 | 5.4 | 1.2 |
| 6 | 5.2 | 1.2 |
| 7 | 5.0 | 1.3 |

*Analysis of variance*

| Variance attributable to: | D.f. | Mean square | |
|---|---|---|---|
| | | Panel I | Panel II |
| Samples | 2 | 33** | 3.7** |
| Temperature | 3 | 1.3* | 1.2** |
| Time | 6 | 0.52 | 0.17 |
| Samples × temperature | 6 | 0.20 | 0.61** |
| Residual | 66 | 0.44 | 0.078 |

* *Exceeds the 5% level of statistical significance.*
** *Exceeds the 1% level of statistical significance.*

The peroxidase value (6) was the time in seconds required for the fluorescence of the milk powder and test solutions to be blanked out by the development of the starch–iodine-blue colour. Milk powder (0.100 gm.) was thoroughly mixed in a fluorometer tube with 8 ml. of solution made from 20 ml. of 0.05 *N* sodium thiosulphate solution and 4.5 gm. of potassium iodide made up to 1 litre with acetate buffer (445 ml. of 0.1 *N* acetic acid and 545 ml. of 0.1 *N* sodium acetate solution). To this, 1 ml. of 2% starch solution was added, followed by mixing; the test-tube was then inserted into the photofluorometer and 1 ml. of 1.0% hydrogen peroxide added from a quick-flowing

blow-out type graduated pipette. A stop watch was started immediately the last drop of hydrogen peroxide was added and stopped when the photofluorometer needle reached 10; the mixture was stirred throughout this period.

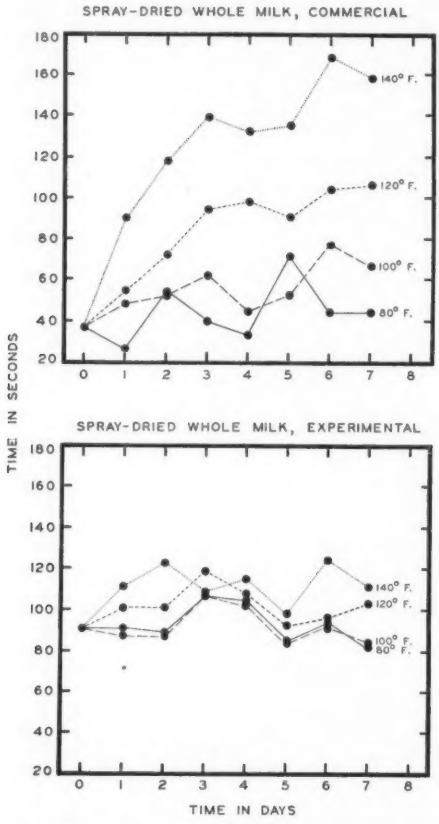


FIG. 2. *Changes in peroxidase activity of commercial and experimental preparations of dried whole milk powders stored for seven days at different temperatures from 26.7° to 60.0° C. (80° to 140° F.).*

*Results*

The results of the taste panel measurements are shown by a table of means and an analysis of variance in Table II. A large difference was evident between the quality of the whole and skim milk, the latter being almost unacceptable to the taste panel. This might be expected for two reasons; the tasters were not accustomed to milk in this form and commercially dried skim milk is usually prepared at temperatures still higher than those used for whole milk. The panel showed a slight preference for the experimental material prepared at low temperatures, although this difference was not

statistically significant. Both panels preferred samples after storage at about 37.8° C. verifying previous similar observations (18, 23). The experimentally prepared powder stored at 60.0° C. and 48.9° C. was of lower quality than the commercially prepared sample and was of much higher quality at 37.8° and 26.7° C. This difference attained statistical significance as noted by Panel II. These results indicate that cooling the powder to 37.8° C. subsequent to drying is desirable. Since time effects were not significant (due probably to day-to-day variation in judgment of the tasters) it is difficult to specify any time within which cooling should be done. Work on dried egg powder indicated that cooling of that product should be done in less than three hours (21); current commercial practice has reduced this time to the order of minutes.

The low average score assigned initial powders by Panel II appeared to be significant. It would appear that even a day's storage at about room temperature improves the palatability of skim milk powders. This phenomenon is currently receiving attention in these laboratories.

Changes in the peroxidase value of dried whole milk powders are shown in Fig. 2. Although a constant peroxidase value had been observed in the initial experiments and a constant value was obtained for skim milk powders, the values for these whole milk powders showed a decrease in peroxidase activity with both time and temperature and the differences were most pronounced in the commercially prepared sample. Similar behaviour had been observed previously (5).

While no justification for cooling subsequent to drying should be drawn from peroxidase values they offer points of interest in continuing the investigation. It is possible that the poor palatability of the samples at 26.7° C. was the result of the action of peroxidase or other enzymes, and the poor palatability at 48.9° and 60.0° C. the result of temperature effects. The better quality of powders stored at 37.8° C. may then be the result of destruction of peroxidase or other enzymes at a rate sufficiently rapid to prevent the enzyme from causing any appreciable deterioration in the powder, while the temperature is still low enough to prevent marked heat deterioration effects. Further attention is being given to the role of enzymes in deterioration of milk powder quality.

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